1-1-1982

The response of membrane potential to low temperatures in a chilling-sensitive and a chilling-resistant plant species.

Kathleen Marie Montaigne
University of Massachusetts Amherst

THE RESPONSE OF MEMBRANE POTENTIAL TO LOW TEMPERATURES IN A CHILLING-SENSITIVE AND A CHILLING-RESISTANT PLANT SPECIES

A Dissertation Presented

By

Kathleen Marie Montaigne

Submitted to the Graduate School of the
University of Massachusetts in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

September 1982

Plant and Soil Sciences

Supported by the

Massachusetts Agricultural Experiment Station

The Response of Membrane Potential to Low Temperatures
in a Chilling-sensitive and a Chilling-resistant
Plant Species

A Dissertation Presented

By

Kathleen Marie Montaigne

Approved as to style and content by:

Paul H. Jennings, Chairperson of Committee

Herbert V. Marsh, Member

Bernard Rubinstein, Member

Terry A. Tattar, Member

Allen V. Barker, Department Head
Department of Plant and Soil Sciences

DEDICATION

This work is dedicated to the unicorns who helped me get through the hard times and to the people who taught me how to see the unicorns.

ACKNOWLEDGEMENTS

I would like to thank Dr. Paul H. Jennings for serving as the chairman of my dissertation committee and for the help he provided along the way. The advice and support of Dr. Herbert V. Marsh, Jr., Dr. Bernard Rubinstein, and Dr. Terry A. Tattar, as members of my dissertation committee, was also appreciated . Special thanks go to Dr. Tattar for the generosity he showed in allowing me the use of his laboratory facilities for three years. Finally, I would like to thank Dr. William Clapham and Wesley Autio for advice on statistics and computer programs.

ABSTRACT

The Response of Membrane Potental to Low Temperature

in a Chilling-sensitive and a Chilling-resistant

Plant Species

(September, 1982)

Kathleen Montaigne, B.S., Southern Connecticut State College

M. S., Syracuse University, Ph.D., University of Massachusetts

Directed by: Professor Paul H. Jennings

The effects of low temperature on the transmembrane potential of maize and oat leaf cells were observed in order to determine whether these effects are related to the degree of chilling sensitivity of the two species, and to gain additional information about the regulation of the membrane potential in higher plants.

At a low light intensity the membranes of both species become less polarized as the temperature is lowered. Although at most temperatures studied the membrane potential of oats was more negative than that of maize, the depolarizing effect of low temperatures was nearly the same in both species.

The transient changes of the membrane potential induced by sudden increased illumination were also studied, and two major differences were found between the two species in the effects of low temperatures on these transients. First, the maximum hyperpolarization of the membrane potential at the higher light intensity was not affected by temperatures above 5 C in maize, while in oats there was a strong decrease in the

maximum hyperpolarized membrane potential value between 9 C and 13 C. Second, the time span over which the transient changes take place was greatly increased in maize at temperatures below 11 C, while in oats there was very little effect of low temperatures on this parameter.

It is suggested that these results can be used as evidence of a voltage-regulated electrogenic pump in higher plants. If this is true, the differences in the chilling sensitivity of the species may be related to a change in the voltage-regulation of the pump in oats at low temperatures which would serve as an energy-conserving mechanism, and to the absence of such a change in maize.

LIST OF FIGURES

LIST OF TABLES

TABLE OF CONTENTS

C H A P T E R I

INTRODUCTION

The cultivation of plants has made possible the selective production of crops, often ones which are not native to the areas in which they are now cultivated. Still, there exist many evolutionary constraints on plant growth, and cultivation practices can not always overcome environmental factors fully enough to grow a plant in an area other than the one for which it is adapted. Because it is necessary for an expanding human population to optimize its usage of agriculturally available land, and because of the economic advantages of certain select crops, there has been a continuous effort on the part of agriculturalists to overcome these innate environmental constrainsts either through control of the environment or through genetic alterations of the plants.

Temperature is one of the crucial environmental controls of plant growth. Only highly adapted plant species can grow in regions of extreme high or low temperatures. Even in between the extremes there is a wide temperature range, and each plant type tends to have a fairly narrow temperature optimum for growth. In temperate zones, the cold temperatures during part of the year prevent the growth of some tropical species entirely, and shorten the growing season of many others. Although freezing temperatures are a major problem in these areas, low but above freezing temperatures also damage a large number of plant species. Such damage is termed "chilling injury" and is mainly of

concern in the spring, at the beginning of the growing season. Low night temperatures at this time of year can have severe effects on the growth of some plants and cause visible damage to leaves and fruit which lowers their economic value. The degree to which a particular species is affected by chilling temperatures varies widely. Plants which are severely damaged by chilling temperatures are deemed "chilling sensitive"; those which are more tolerant of low temperatures, and which may exhibit no marked low-temperature response above freezing temperatures, are deemed "chilling resistant", or "chilling tolerant".

Determination of the primary mechanism(s) of chilling injury, and particularly of the basis of the differences in temperature response between sensitive and resistant species, would greatly facilitate the efforts of agriculturalists in their attempts to overcome the problem of chill-sensitivity. Therefore, a number of hypotheses have been formulated and tested with varying degrees of success. Although in many cases considerable evidence has been collected in favor of a particular hypothesis, the issue has yet to be clearly resolved.

It has been suggested that membranes play an important role in chilling injury. Since membrane potential is a function of plasma membrane properties, and is involved in the regulation of many physiological processes, it is a logical candidate for the site of a primary low temperature effect which could produce a variety of symptoms. The purpose of the work presented here is to look at the difference between maize (a chilling sensitive species) and oats (a chilling resistant species) in the response of membrane potential to low

temperatures, both to determine if this response is related to temperature sensitivity and to gain additional information about the control of membrane potential in higher plants.

C H A P T E R II

LITERATURE REVIEW

Chilling Injury

Plants are susceptible to damage from low temperatures at various stages of their development, including seed imbibition and germination, early seedling growth, flowering, and fruiting. Both the fruit and the vegetative organs may be affected, and the injury may show up in different forms.

In fruit, the common visible symptoms are surface pitting, development of necrotic areas, external discoloration, or a failure to ripen. In seedlings, injury is likely to be expressed as a susceptibility to decay, appearance of chlorotic and/or necrotic lesions, or a reduction or cessation of growth (77). The subsequent discussion of chilling injury will focus primarily on effects on young seedlings.

Although visible symptoms are an important factor in determining the economic significance of chilling injury, work aimed at improving the resistance of plants to low temperatures will depend on knowledge of the physiological bases of these symptoms. In order for observations of the physiological effects of low temperatures to be useful in determining the primary mechanisms of chilling injury it is necessary either that the effects be correlated to the known temperature of chilling injury in a particular species, or that differences in the temperature response

between species be related to differences in their chilling sensitivity.

Determining the primary response(s) to low temperature which can be demonstrated to be the basis of the wide range of observable effects is even more important than studying the effects of low temperature on individual physiological processes. Therefore, although many physiological and metabolic processes have been observed to be affected by low temperature, including protoplasmic streaming (66,97), solute leakage (19,96,100,144), respiration (24,75,141), photosynthesis (25,26,95,110), and the activity of a variety of enzymes (77); not all of this information has contributed to a clear-cut theory of chilling injury.

One group of workers has proposed that the primary effect of low temperatures is the creation of a water stress, which in turn is responsible for the many symptoms of chilling injury (43,143). According to this theory, low temperatures cause either increased water loss from leaf cells (44,144) or increased root water resistance (26,56). Support for the hypothesis grew from the observations that chilling resistance can be at least partially induced in some sensitive species by a prior drought treatment, and that elimination of water effects via maintenance of an atmosphere of high relative humidity can often reduce the symptoms of chilling (44,141,143,144). However, elimination of water stress during chilling treatments does not eliminate all of the chilling symptoms (30,144) and in some species water stress does not seem to be involved (141). Also, the involvement of water stress interactions still requires a search for a primary

mechanism by which low temperatures lead to development of the water stress.

A second suggestion has been that chilling injury is directly related to changes in cell membrane permeability. This can be related to the induction of a water stress, since changes in membrane permeability could decrease the uptake of water by roots, and water flow through the whole plant (56). Aside from dehydration effects, changes in membrane permeability could result in increased solute leakage from cells which would cause metabolic changes.

Evidence for low temperature changes of membrane permeability is primarily derived from studies of solute leakage from chilled tissues. Although increased leakage has often been observed in chilled tissues, many of these studies measured leakage only after the chilling treatment was completed, and the chilling was often at very low temperatures and of long duration (24,44,89,143,144). This type of study provides little evidence of a direct primary effect of low temperature on membrane function, although it can often be used to assay the extent of injury in a chilled plant. When leakage was studied as an after effect of chilling in leaves of cucumber (89,144), cotton (44), corn (24), or beans (143), or roots of cotton (19), loss of electrolytes (24,44,89,143,144), carbohydrates (19,44), and amino acids (19,44) was seen to increase with decreasing temperature and increasing duration of treatment. The effect was reversible for mild treatments, but recovery was prevented if the temperature was very low or the exposure was prolonged (24,143,144). Membrane permeability was followed from the

onset of chilling in algae (54), and in different altitudinal species of Passiflora (95) and Lycopersicon (100) In the alga Chara corallina Hope + Ascherger (54) showed, using calculations based on measurements of membrane potential and resistance, that the permeability of the plasma membrane to both Na^+ and K^+ decreased constantly and equally for both ions with decreasing temperature down to 7 C. Below 7 C, the permeability to K^+ decreased more rapidly than the permeability to Na^+. In the alga Griffithsia pulvinata the permeability to K^+ was decreased more rapidly than the permeability to Na^+ over the entire temperature range studied. Using Passiflora species, Patterson et al. (96) observed a slow rate of solute leakage, primarily K^+, within the first few days of chilling at 0 C, followed by a faster leakage rate which occurred at about the same time as visible lesions on leaves became apparent. The number of days at 0 C before the second leakage stage was reached was greater in species known to be tolerant of cool temperatures than in the more sensitive species. Paull (100) found changes in rates of leakage of leucine and rubidium with chilling temperature within one hour from the onset of temperature treatment. Using chilling-sensitive species, leakage of both substances was shown to decrease with decreasing temperature down to 5 - 10 C, and then to increase with further temperature drops. The extent of leakage at the lowest temperatures was roughly correlated to the chilling sensitivity of the tissue. From all of the above evidence it is clear that solute leakage and changes in membrane permeability are indicators of chilling injury; however it is not clear whether these changes are in fact the basic

cause of the damage.

Another suggestion has been that chilling injury is caused by decreased energy availability in the affected tissues. This suggestion was originally based on observation of low temperature effects on respiration (24,75,141), and photosynthesis (26,30,95,110), and corroborated by direct measurements of tissue ATP levels (120). Since one of the major symptoms of low temperature stress is a reduction or cessation of seedling growth, it seems likely that photosynthesis is one of the physiological processes that is strongly influenced by chilling. Still, the effect on photosynthesis can be either a direct one, on photosynthetic enzymes or light reaction processes, or an indirect one, via an effect on stomatal conductance or source-sink relationships. Starch accumulations are seen in the chloroplasts of chilled plants at the end of the dark period, when in non-chilled plants such accumulations have been mobilized. This observation led to suggestions that the primary effect of low temperature is on the systems normally operating to break down starch and transport it's products from the leaves to sites of carbohydrate use. According to this theory, measured reductions in photosynthetic activity are due to the feedback inhibition of CO_2 fixation by the presence of the starch, and are therefore secondary in nature (36,51,126). As evidence against the feedback inhibition theory, accumulation of starch was shown to be ineffective in reducing the photosynthetic rate unless accompanied by cool root temperatures (26).

The response of stoma to low temperatures, and the possibility that

increased stomatal resistance at low temperatures limits the supply of CO_2 available for photosynthesis have also been investigated. Several workers have observed reductions in net photosynthesis which correlated with increases in stomatal resistance during and after a period of chilling, and have assumed that stomatal closure was the cause of reduced photosynthesis (26,43,95,128). But Drake and Raschke (30) showed that the concentration of CO_2 within the stomata was high, which would suggest that availability of CO_2 was not the limiting factor. Also, Martin + Boyer (82) were unable to eliminate the low temperature effect on photosynthesis by maintaining a saturating intercellular CO_2 concentration.

Definite effects of low temperature on light reaction processes (25,35,82,83,91,92,113,114), and on enzymes of the dark reactions (26,41,85,127,134) have been seen, although the relationship of these effects to chilling sensitivity are unclear. Garber (35) described irreversible low temperature inactivation of thylakoids, as determined by measurements of proton uptake, osmotic response, and phosphorylation, which occurred only when high intensity light exposure accompanied the chilling treatment. Loss of electron transport ability has also been seen as an after effect of low temperature treatment (83,114), and correlated to chilling sensitivity (113). In studies performed during the chilling treatment, electron transport and photoreduction were again affected. In some cases the response was the same in sensitive and resistant species (25,92), while in others the degree of response could be correlated to chilling sensitivity (113). Similar contradictions

were found when the activities of PEP carboxylase (41,85,134), RuBP carboxylase (26,134), and other photosynthetic enzymes (26,127) were studied as a function of temperature. Again, some workers were able to correlate temperature sensitivity to the extent of the temperature effect on these enzymes, while others saw the same response in both sensitive and resistant species.

Respiratory changes were among the first physiological responses to chilling which were studied. Most of the early observations, however, were made on fruits after long periods of post-harvest storage (77), and will not be discussed here. In work with seedlings, it was shown that in most species respiration decreases with temperature (1,10,11,12,89,141), and that the effect is reversible after chilling episodes of short duration (144). Creencia + Bramlage (24) reported increased C_2 consumption using chilled (0.3 C) maize seedlings, as well as a decreased sensitivity to DNP which suggested an uncoupling of oxidative phosphorylation; this effect was also reversible upon return to room temperatures. In the extremely chill-sensitive plant, Episcia reptans , respiration increases drastically during the first 80 min. of exposure followed by a gradual decrease thereafter, suggesting the possibility of a separate mechanism of injury in plants with such high sensitivity (141). Although decreases in respiratory activity were observed in both sensitive and resistant species, differences in the degree of the low temperature response have been found (15,75).

A few attempts have been made to look directly at the energy levels maintained in chilled tissues, since one result of altered

photosynthetic or respiratory rates would be changes in energy availability. Stewart + Guinn (120) observed decreases in ATP supply at 5 C in both leaves and roots of cotton seedlings, although in roots the eventual decline was preceded by an initial increase. ATP levels increased during hardening of the seedlings, and continued to increase in the leaves of hardened plants which were subjected to 5 C, while decreasing in the roots of these plants. This gave strong support to the hypothesis that the symptoms of chilling injury are the result of metabolic disruption mediated by tissue energy supplies, but contradictory evidence has also been collected. In several species (58,126) it appears that the immediate effect of chilling temperatures is an increase in ATP, followed by a decrease (126) which shows up at approximately the same time as visible lesions. Wilson (141) saw an immediate drop in ATP supply of Episcia at 5 C, but there was only a 25% loss within 5 hours at which time there was 100% leaf injury; using Phaseolus , he showed that falling ATP levels were correlated with a chill-induced water stress rather than with chilling temperatures alone. These results suggest that reductions in available ATP are an after effect of chilling injury, rather than the cause. However, interpretation of any of this evidence is complicated by the lack of information on individual pools of ATP. Changes in tissue ATP levels tell little about the amount of ATP actually available for fueling metabolism.

With all the different physiological processes which can be seen to be adversely affected by low temperature, and with the differences in

the responses of plants having similar chilling sensitivities, it is difficult to imagine a single mechanism which can be identified as the cause of sensitivity. Nonetheless, a mechanism has been suggested which may be able to explain both the variety of observable symptoms and the differences between species. Lyons, et al. (73) considered the possibility that the mitochondrial membranes of chilling-sensitive plant species might be less flexible than those of chilling-resistant plants, in analogy to findings with animal cells (111). Although original results were inconclusive, some differences were seen in the fatty acid composition of mitochondrial membrane lipids of sensitive and resistant species. Later, Lyons and Raison (75) suggested that these differences in lipid composition might be responsible for different temperatures of phase transition of mitochondrial membranes from a liquid-crystalline to a solid state; a phenomenon shown (72,74) to be strongly responsive to small changes in fatty acid saturation. In this scheme, which has been greatly extended and modified since first being proposed, changes in phase of membrane lipids are suggested to cause changes in the functioning of membrane bound enzymes via conformational changes or altered interactions with nearby molecules. This may explain the observation (105) that the activation energies of several otherwise dissimilar mitochondrial enzymes are greatly increased below a single critical low temperature, in some chilling sensitive species.

Since the activation energy of an enzyme or reaction can be determined from an Arrhenius plot of its rate, such plots have been used in studies of temperature sensitivity to identify reactions or processes

which exhibit changes in activation energy at critical chilling temperatures (76). Other tools which have been used in testing the phase transition hypothesis include chemical probes which pick up changes in the molecular ordering of the lipids into which they are incorporated (94,98,104), and analyses of the fatty acid composition of membrane lipids which show differences in fatty acid composition of sensitive vs. resistant plants and unhardened vs. hardened plants (98,104).

Changes in the slope of Arrhenius plots, indicating changes in activation energy, have been found for respiration (10,11,15,75,89), photosynthetic light (92,113) and dark (41,85,127) reactions, chloroplast permeability (91), growth (108), non-mitochondrial ATPases (64,84), and ion uptake (15,99). In some of these cases, changes in activation energy at chilling temperatures were seen in chilling-sensitive plants, but were absent for chilling-resistant plants (41,75,91,99,113). However, in other studies chilling-resistant plants also exhibited breaks in Arrenhius plots of some characteristics (84,92).

Strong support for the phase transition theory of chilling injury is provided by studies which have correlated the temperature of changes in enzyme activity with the temperature at which changes in molecular ordering of membrane lipids occurs (98). Similarly, a high degree of unsaturation of fatty acid components of membrane lipids, and therefore a lower temperature at which phase transition would be expected to occur, can be found in resistant or chill-hardened plants as compared to

unhardened sensitive species, in at least some cases (63,73,98,104).

The fact that some resistant plants are similar to sensitive plants both in their fatty acid composition and in the response of some of their physiological processes to low temperature, suggests that resistance may be maintained either by the avoidance of the low temperature phase transition, or by increased tolerance to the effects of such transitions (88,140).

The closer study of lipid transitions which has been made since the initial hypothesis was developed has shown that the concept of a single phase transition at a particular temperature is an oversimplification of the process. In a mixture of lipids, as found in most membrane systems, different lipids will change phase at different temperatures (69,145). The observable effects on enzymes are probably related to the phase transition of those lipids in their immediate vicinity, or to regions of instability which occur at the boundaries between lipids in a solid and a fluid state (104). Since it is also true that different membranes (mitochondrial, chloroplast, plasmalemma) have different lipid compositions, there are many possible sites of chilling sensitivity. This is useful in explaining the different responses to chilling in various species, including separate effects on respiration, photosynthesis, and cell leakage; but complicates the study of the phenomenon.

Membrane Potential

Generation of the membrane potential. The existence of electrical gradients across membranes is a well-documented phenomemon and is commonly observed in plant cells (27,47,49). Although transmembrane potentials are known to form across the tonoplast, mitochondrial membranes, and chloroplast membranes, this discussion will focus primarily on the membrane potential (Em) across the plasmalemma of plant cells. In higher plants, plasmalemma potentials of - 100 mv to - 200 mv, interior negative, have been reported.

The occurrence of membrane potentials depends in part upon the permeability characteristics of membranes. Since biological membranes are differentially permeable, various ions are transported across membranes at different rates. Depending on the charge of the ions involved, this can lead to a separation of charge across the membrane. A membrane potential generated in this way is known as a diffusion potential, since diffusion is the driving force behind it's development and metabolic energy is involved only indirectly, in the maintenance of membrane integrity.

In some cases, a pool of non-diffusible charged molecules creates a special type of diffusion potential known as a Donnan potential (46). For instance, the cytoplasmic proteins of most cells are strongly negatively charged and are much too large to diffuse freely across the plasmalemma. Small, positively-charged ions diffuse into the cells along the electrical gradient created by the negatively-charged

proteins; but eventually the force of the concentration gradient pulling these ions out of the cell equals the force of the electrical gradient pulling them in. At this point a thermodynamically stable electrochemical equilibrium is established, although there is still an electrical gradient across the membrane. Again, a Donnan potential is a passive phenomenon, since there is no net movement of ions against the electrochemical gradient.

Since diffusion potentials are thermodynamically passive, it is theoretically possible to calculate their magnitude using various equations. The Nernst equation (Eq. 1) can be used to calculate the diffusion potential which would be created by a single ion species.

EQUATION 1: $$E_j = \frac{RT}{z_j F} \ln \frac{a_j^o}{a_j^i}$$

where R= the gas constant, T= the temperature, z =the charge on the ion, F=Faraday's constant, and a_j=the activity of ion j in (i) or out(o) of the cell.

Since total membrane potential is dependent on the interaction of a number of ionic species, and their different permeabilities, the Goldmann equation (Eq. 2) attempts to describe the membrane potential which would arise, assuming that K^+, Cl^-, and Na^+ are the major ions involved (40).

EQUATION 2: $$E = \frac{RT}{F} \ln \frac{P_K a_K^o + P_{Na} a_{Na}^o + P_{Cl} a_{Cl}^i}{P_K a_K^i + P_{Na} a_{Na}^i + P_{Cl} a_{Cl}^o}$$

where P_K, P_{Cl}, and P_{Na} are the permeability coefficients of the three ions.

However, even the Goldmann equation is an oversimplification of the situation existing in most cells, and may neglect important ions (62), such as H^+. Still, it provides the opportunity to calculate at least rough estimates of the magnitude of diffusion potential which can be expected to form under set conditions, and to predict the effect of changes in the ion concentrations.

Contrary to the views of early workers in plant membrane transport, it now appears that the membrane potential of most cells is not generated by diffusive forces alone. As early as 1935 (9) there was evidence that there is also an active component of the membrane potential, i.e. a system or systems which move charge across the membrane against an electrochemical gradient, at the expense of metabolic energy. Transport systems which are involved in the movement of charge across the membrane resulting in an increased electrical gradient are known as electrogenic pumps.

The two major lines of evidence which support the theory of an active component of the membrane potential are 1) that the measured Em is, in some instances, more negative than can be accounted for by calculation of the expected diffusion potential (18,23,40,48,101,116), and 2) that the membrane potential becomes depolarized in the presence of metabolic inhibitors (14,16,23,39,40,59). Although the evidence of the existence of electrogenic pumps in plant cells is extensive, which ions are being transported and the extent to which active processes contribute to the total membrane potential vary among species and tissues.

Ion pumps of H^+, K^+, Na^+, OH^+, P_i^-, and Cl^- have all been postulated to be present in some plant tissue (2,3,4,17,22,37,67), although not all of these pumps are necessarily electrogenic. Ion stimulated ATPases bound to non-mitochondrial membrane fractions (3,6,20,21,22,34,37,52) have provided further evidence that some of these ions may be actively transported at either the tonoplast or plasmalemma, as well as supporting the common belief that ATP serves as the major energy source for active transport. Proton pumps are most often implicated as the primary origin of electrical gradients (102). Stimulation of proton extrusion by auxins or by the fungal toxin fusicoccin have shown that proton movements can be of significant magnitude and may be important for some physiological processes (80). Several reports have provided evidence of co-transport of protons with carbohydrates or amino acids, with an electrochemical gradient of H serving as the driving force (32,78,135). Recent work with sealed membrane vesicles of non-mitochondrial origin have shown that ATPases within these membranes do act as electrogenic proton pumps (7,20,21,31,121,124). However, it is possible that these pumps are associated primarily with the tonoplast and/or endoplasmic reticulum (20,21,79) and that they cannot be strictly correlated with development of the Em across the plasmalemma.

The effect of light on the membrane potential. Light has been shown to cause fluctuations of the membrane potential in green cells of many species, but the nature of the changes is variable. In Griffithsia light causes a depolarization of the membrane potential

(130,131,132,133); and in Oenothera , Phaeoceros , and Potamogeton , the resting potential in the light is equal to that in the dark, following initial transient changes (13,23,29). However in most species studied the membrane potential becomes hyperpolarized in the light (8,33,39,40,57,103,112,115,137) although the final hyperpolarization may be preceded by an initial depolarization (8,33,57,103,115,118,137). Luttge and Higinbotham (71) suggest that in many of the instances where a light-induced hyperpolarization has been reported, the hyperpolarization may be temporary - part of a series of oscillations of the Em which continues with decreasing amplitude and increasing period until a steady-state Em is established which is equal to that seen in the dark. Still the hyperpolarization in Acetabularia (40,112), Vallisneria (8,103), Elodea (57), and Nitella (115,118) has been observed over long time periods.

Steady-state hyperpolarization of the membrane potential in the light has been attributed to an increased energy availability during photosynthesis. This response has been shown to be eliminated by conditions which prevent light-dependent ATP production (57,103,112,115) but maintained under conditions which prevent CO_2 fixation without affecting ATP production (103,115), lending support to the theory that ATP serves as the metabolic link between photosynthetic activities and the membrane potential.

The basis of the light-induced transient changes in membrane potential is less clear. A common explanation of the initial depolarization of Em is that it is a reflection of an increase in the

cytoplasmic pH which would decrease the contribution of H^+ to the diffusion potential. Attempts have been made to correlate the Em changes with transient changes in cytoplasmic pH (28,71,93). Reasonable correlation between the two has been found in Atriplex (93) and Zea (71), but in Phaeoceros (28) the correlation holds for the light to dark transients but not for the dark to light transients. The cause of the transient light-induced cytoplasmic pH change is unknown in view of the apparently low H permeability of the chloroplast envelope, although it has been suggested that the pH changes are brought about by temporary changes of the ATP:ADP ratios in the cytoplasm (71,138). A second explanation of the transient changes has been provided by Vredenberg + Tonk (137), working with Nitella , and Bentrup, et al. (8), working with Vallisneria. They believe that an unidentified trigger turned on by light causes an immediate increase in the permeability of the plasmalemma to H^+, leading to a depolarization of the diffusion potential. The subsequent repolarization and hyperpolarization occur as increased energy supplies power the electrogenic pump.

The effect of low temperatures on the membrane potential. Low temperatures have been shown to cause a depolarization of the membrane potential in a number of studies. In most of these studies a single low temperature has been used to induce depolarization, and the response has been attributed to either an increase in membrane permeability (53,54) or an inhibition of an electrogenic pump, either directly or through a decrease in the metabolic energy supply (39,40,87,112).

Several workers have made more detailed analyses of the effects of low temperatures on the membrane potential. Spanswick (115) showed that the response of Em to decreasing temperature in Nitella was ~ 2.5 times as great in the light as it was in the dark, and that low temperatures cause a proportionally greater increase of membrane resistance in the light than in the dark. Using Oenothera , Melamed-Harel and Reinhold (86) found a pattern of decreasing membrane potential and increasing membrane resistance with decreasing temperature and a hysteresis of both responses upon rewarming which suggested an effect of lipid phase transitions on an electrogenic pump and separate effects on membrane permeability. In oats (55,61) and maize (55) low temperatures were shown to affect solute-induced changes in membrane potential, which again were interpreted as effects on the electrogenic activity of the tissues. In both maize and oats low temperatures decreased the rate of recovery following the depolarization of the Em caused by addition of sugars or amino acids to the external medium, or by sudden illumination. However the low temperature effect was more pronounced in maize, and this was attributed to the greater chilling sensitivity of this species (55). Bravo-F. and Uribe (10) looked in detail at the effect of low temperature on respiration and on the Em of maize root cells and concluded that the Em response was greater than could be accounted for by an effect of temperature on the respiratory energy supply, and was likely to be related to a more direct low temperature effect on the electrogenic pump(s). Most recently Nelles and Laske (90) reported a strong depolarizing effect of low temperature on the Em of rapidly

cooled maize coleoptile cells, and a lesser depolarizing effect when the cells were slowly cooled. They suggest that slow cooling allows for changes in the lipid composition of plasma membrane, which in turn allow for increased electrogenic activity. However their results are subject to other interpretations, as will be discussed below.

C H A P T E R III

MATERIALS AND METHODS

Plant Material

All experiments were carried out using seedlings of *Zea mays* cv Agway 590X and *Avena sativa* cv Garry. Seeds were planted in vermiculite and watered with a modified Hoagland's solution, containing three times the normal amount of chelated iron. Flats of plants were kept in a Warren-Sherer growth chamber with incandescent and cool-white fluorescent lights providing a light intensity of 30 W/m^2 during the day. A 15/9 hour day/night light regime was maintained, at a constant temperature of 24 C.

Preparation of Leaf Sections for Membrane Potential and Proton Efflux Measurements

The day before any measurements were made, the first leaf was removed from 7 - 14 day old seedlings. The lower epidermis was removed from these leaves using forceps, to facilitate both ion exchange between the mesophyll cells and various bathing mediums, and puncture of these cells by microelectrodes. Segments approximately 2 x 10 mm in size were taken from the regions on either side of the leaf midrib, between 1 and

5 cm from the leaf tip in maize, and between 1 and 8 cm from the leaf tip in oats. Leaf segments were placed, stripped side down, in petri dishes containing approximately 40 mls of a bathing solution (see below). Tissue was maintained in this way, in a growth chamber at 23 C, until the following day when various characteristics were studied. Increased rates of ion transport are seen in tissue which is "aged" in this manner, but it is unclear whether the aging process merely allows for restoration of transport rates disrupted by excision, or induces the development of rates which would not be maintained in undisturbed tissue (42,68,136).

Membrane Potential (Em) Measurements

Leaf segments prepared and preincubated as stated in the previous section were placed, stripped side up, in a plexiglass tissue holder, which in turn was placed in a plexiglass perfusion chamber (Fig. 1). Perfusion solutions entered the chamber through Tygon tubing connected to a solution reservoir. The rate of flow of solution from the reservoir was regulated by a valve on the tubing, thus providing variable perfusion rates of solution over the leaf tissue.

Micropipettes were pulled from capillary tubing containing an extruded fiber and with an outside diameter of 2 mm (Frederick Haer + Co.), using an Industrial Science Associates horizontal, or David Kopf Instruments Model 700 C vertical, micropipette puller. Micropipettes were trimmed to a shank length of ~ 15 mm and filled with 3 M KCl.

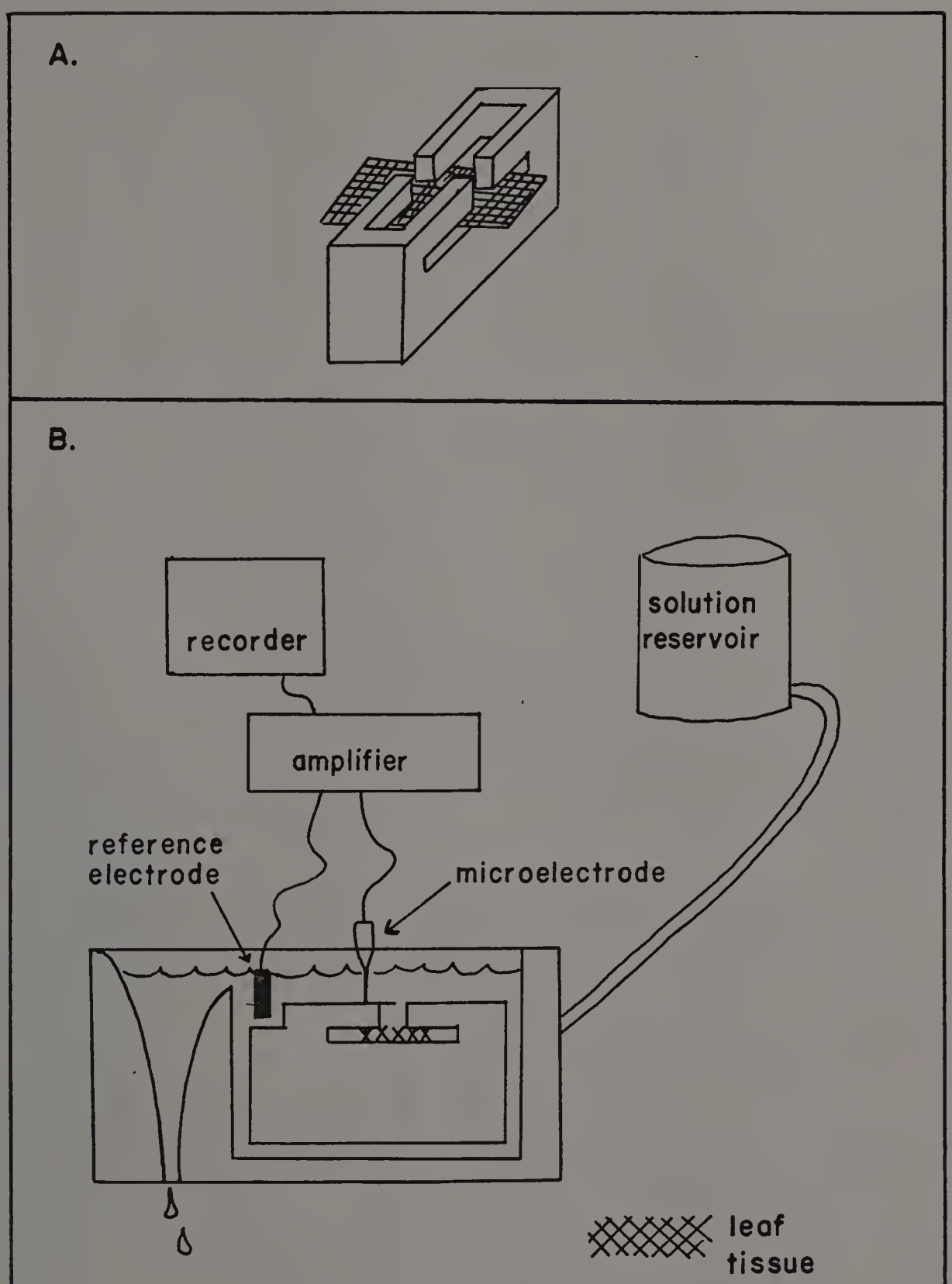

Fig. 1. Experimental system for electrophysiology

A. Tissue chamber

B. Perfusion and measuring system

Adjustments to the micropipette pullers were made so that the resulting microelectrodes had resistances of 20 - 60 M , with an average of about 25 - 30 M , since such electrodes gave the best results. The microelectrodes were stored until use in a solution of 3 M KCl. For use, a microelectrode was placed in a microelectrode holder which was then put in place in the experimental set-up (Fig. 1). The reference electrode consisted of a plug from a 2% agar plate made to 3 M KCl and held by Tygon tubing,and was placed in the perfusion chamber. Both electrodes were connected to a W. P. Instruments Model 725 microprobe amplifier with Ag - AgCl leads.

When the tissue, electrodes, and perfusion solution were all in place, the microelectrode was lowered to the tissue with micromanipulators. With the microelectrode situated just above the tissue, the fine control knob of the micromanipulator was used to probe individual plant cells. The electric potential difference between the microelectrode and the reference electrode was recorded on an Esterline Angus Miniservo chart recorder. In higher plant cells it is generally assumed that the electrode passes through the thin cytoplasmic layer and rests in the vacuole during Em measurements, so that the electrical potential being measured is actually that which exists between the vacuole and the external medium (Fig. 2). Although the membrane potential across the tonoplast generally has been thought to be small in magnitude relative to that across the plasmalemma (47), recent work (5) suggests that this may not be the case. Therefore any interpretation of data collected in this way must consider the possibility of effects at

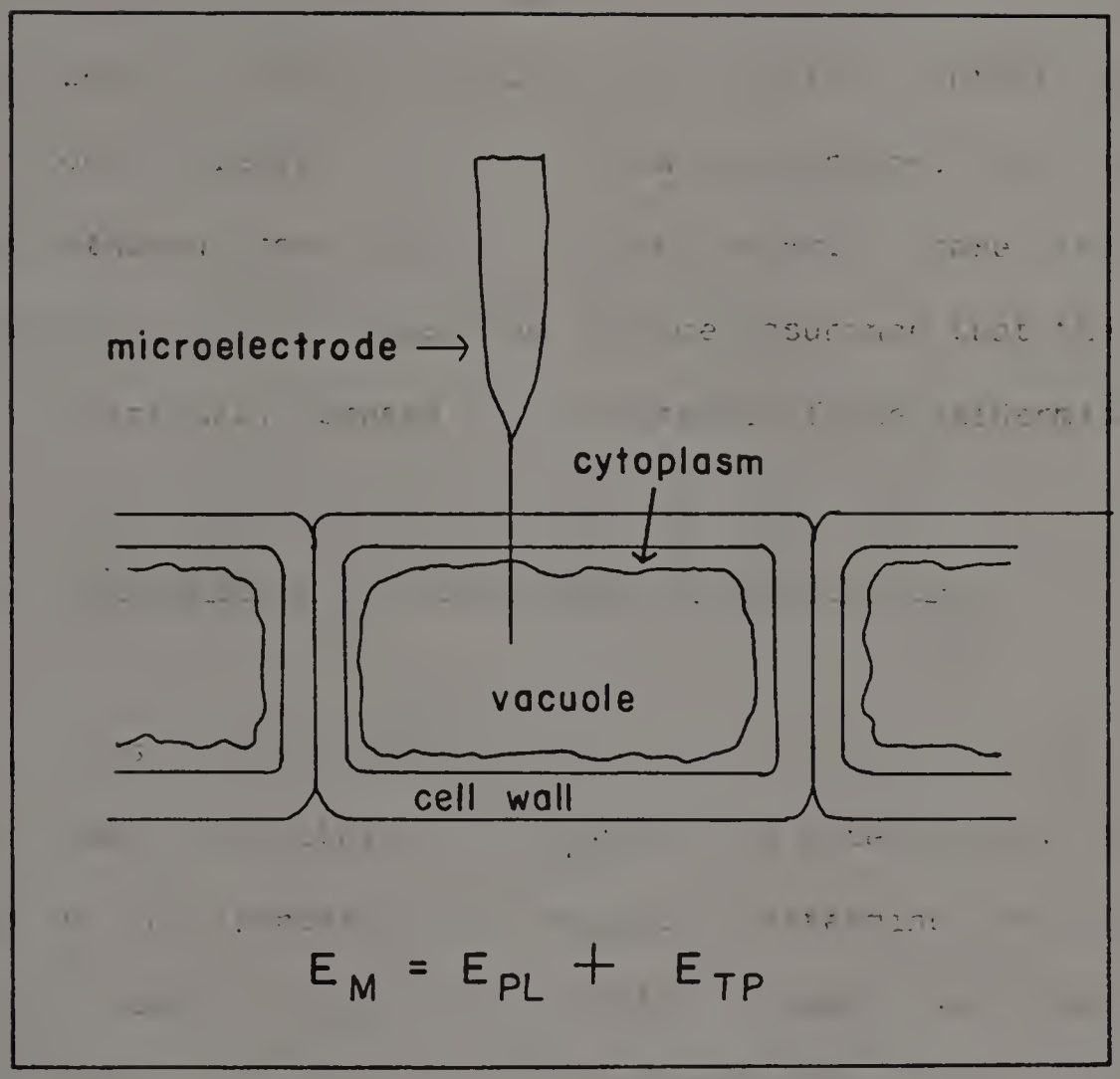

Fig. 2. Microelectrode placement within cell.

Total membrane potential measured is a combination of the potentials across the plasmalemma and the tonoplast.

Values were obtained from one to four cells in each leaf segment, after which a new segment was used. At each of the five perfusion rates (2, 5, 8, 13, and 19 mls/min.) values from 30 cells were recorded.

The Effect of External pH on Em

The effect of pH on membrane potential was also studied. In this case the perfusion and preincubation solutions were composed of 1 mM KCl, 1 mM $CaNO_3$, 0.25 mM $MgSO_4$, and 1 mM MES or 1 mM HEPES, and adjusted to the desired pH value with 1 N NaOH.

The procedure was similar to that of the perfusion rate experiment; however the resting potential was recorded after the value was stable for only 5 minutes. At each pH (5.5, 6.0, 6.5, 7.0) Em values were recorded from 15 cells.

The Effect of Temperature on Em

For this experiment, both maize and oat tissues were used. The preincubation and perfusion solutions were 1 mM KCl, 1 mM $CaNO_3$, 1mM NaH_2PO_4, 0.25 mM $MgSO_4$, and 1 mM MES, adjusted to ph 5.5 with 1 N NaCH.

After the overnight preincubation of tissue in the growth chamber, the leaf segments were transferred to fresh bathing solution in beakers kept in a Forma Scientific Model 2095 refrigerated bath. The tissue was maintained in the dark, and at the temperature to be studied, for at least two hours before transfer to the Em monitoring set-up. Since leaf

segments were removed from the low temperature bath at various times during the day to be used for Em measurements, the duration of the low temperature preincubation varied from 2 - 8 hours.

The perfusion solution was chilled before use until it just began to freeze (~ 0 C). It was maintained at the freezing point in the solution reservoir by surrounding the reservoir with packed ice. The solution warmed up as it passed through insulated tubing between the reservoir and the perfusion chamber, and, by controlling the rate of flow, the perfusion chamber could be maintained at any of the desired temperatures. The temperature of the perfusion chamber was monitored using a Yellow Springs Instrument Co. tele-thermometer, with a YPI series 400 probe placed in the solution adjacent to the tissue. The temperature was checked just before and just after each cell was studied, and in a few cases was monitored continuously to assure that the temperature did not fluctuate during the Em measurements.

For measurements of Em, the leaf segments were transferred to the experimental system in dim light (0.2 W/m^2), and at the same temperature as during the preincubation. After the resting potential was determined at this low light intensity, and while the same cell was still being monitored, the tissue was illuminated using an American Optical Corp. microscope lamp, bringing the light intensity to 3 W/m^2. In this way, a recording was obtained of the resting potential at the lower light intensity, and of the response of the membrane potential to sudden increased illumination.

The typical response to sudden increased illumination was an

immediate partial depolarization of the Em, followed by a repolarization and hyperpolarization. The recording of the response was continued until hyperpolarization was complete. Hyperpolarization was considered to be complete when the hyperpolarized Em value was constant for at least 5 min., or when a slight depolarizing trend was seen.

This process was repeated for 3 - 10 cells at each temperature. Only one cell was used from each leaf segment in order to avoid any affect a single period of illumination might have on subsequent measurements in low intensity light.

The Effect of Temperature on Proton Extrusion from Leaf Segments

In this experiment the extent to which leaf cells were able to acidify the medium in which they were placed was used as a way to monitor extrusion of protons by the cells.

Leaf segments from maize and oats were prepared as for the previous experiments. An overnight preincubation under normal growing conditions preceded all measurements. The preincubation medium was 1 mM KCl, 1 mM $CaNO_3$, 0.25 mM $MgSO_4$, 1 mM NaH_2PO_4, and 1 mM HEPES, adjusted to pH 7.0 with 1 N NaCH. Temperature treatments were provided in growth chambers, either in the dark, or illuminated with incandescent and cool-white fluorescent lights at an intensity of 20 W/m^2. Leaf segments were kept in the preincubation medium at the temperature to be studied for 2 hours after the overnight preincubation at 23 C, and before monitoring of proton movements began.

For measurements of net proton extrusion, leaf segments were placed in 25 ml beakers containing 1 ml of solution, similar in composition to the preincubation medium, but with the concentration of KCl increased to 10 mM and the pH adjusted to 6.5. There were 15 leaf segments/beaker, with each treatment run in duplicate. The pH of the solution was measured with a Fisher Scientific flat-surface pH electrode every 60 minutes for 5 hours. Changes in the pH of the solution were used to calculate the loss or gain of protons, by comparison to a HCl titration curve of the same solution. Because of large daily variation in the activity of the tissue, the results of the low temperature treatment were expressed as a percentage of the activity in a 19 C control, run simultaneously.

C H A P T E R IV

RESULTS

The Effect of Buffer Perfusion Rate on Em

When resting potentials of cells monitored at various perfusion rates (similar to those used in later temperature studies) were measured, values ranging between -80 mv and -135 mv were obtained, with a mean value of -109 mv. The range of values observed at each of the five perfusion rates studied, and the mean value at each rate, are given in Table 1, along with the results of an analysis of variance of these values. No significant effect of perfusion rate on the resting potential of maize leaf cells was seen.

Some cells were monitored continuously as the perfusion rate was dropped stepwise, starting at the highest rate and continuing until all flow was halted. In this way it was possible to look for any transient changes in Em which would not be noticed by measurements of resting potential. There was no observable response to these stepwise changes in perfusion rate, except when the flow was stopped entirely. However when the flow of perfusion solution was stopped the Em became partially depolarized, and when the flow was resumed the Em became repolarized.

It is possible that without solution flow, respiration became rate-limited by the unavailability of oxygen, and the resultant decrease in available metabolic energy inhibited the maintenance of the active component of the membrane potential. Nonetheless, it is clear from both

TABLE 1. The Effect of Buffer Perfusion Rate on Membrane Potential.

Perfusion Rate (ml/min.)	2	5	8	13	19
Range of Values Observed (-mv)	80 - 119	92 - 135	99 - 135	96 - 132	94 - 132
Mean of Values Observed (-mv)	106	111	109	106	114
Standard Error	1.6	1.9	1.5	3.6	1.4

Analysis of Variance

	df	SS	MS	F
Total	149	21355.9		
Perfusion Rate	4	1265	316.25	2.28
Error	145	20090.9	138.56	

the immediate response to stepwise changes, and the observations of resting potential, that there is no such limiting effect at even the slowest of the perfusion rates used in the temperature studies.

The Effect of External pH on Em

When the resting potentials of maize leaf cells bathed by solutions varying in pH from 5.0 to 7.0 were measured, Em values ranging from -100 mv to -126 mv were observed (Table 2). An analysis of variance of the results shows that there is no significant effect of the external pH on the resting potential of maize leaf cells, within the pH range studied.

Transient changes in Em in response to sudden changes in external pH were also studied. After the resting potential was measured at one pH and, while continuously monitoring the Em of the same cell, the perfusion solution was replaced with a second solution at a different pH. Measurements were continued until a steady Em was maintained for at least five minutes, which was taken as an indication of the re-establishment of a resting potential. Typically, when the pH change was from 5.5 to 7.0 there was an immediate depolarization of the membrane potential followed by a gradual repolarization and return to a resting potential similar to that seen before the pH change. When the perfusion solution was changed back to pH 5.5, the opposite response was seen. In this case an initial hyperpolarization was followed by a gradual depolarization which resulted in a final resting potential very close to the initial value. A lag period of ~ 1 minute before the Em

TABLE 2. The Effect of External pH on Membrane Potential

pH	5.5	6.0	6.5	7.0
Range of Values Observed (-mv)	101 - 126	110 - 118	100 - 118	100 - 123
Mean of Values Observed (-mv)	112	115	108	113
Standard Error	2.2	0.9	1.6	2.1

Analysis of Variance

	df	SS	MS	F
Total	39	1366.4		
pH	3	251	83.67	2.7
Error	36	1115.4	30.98	

showed a response to each switch of perfusion solution is equivalent to the time it took for the new perfusion solution to reach the chamber in which the tissue was held.

It is apparent from these results that pH changes do have a temporary effect on membrane potential, but that mechanisms within the cells allow them to adjust to the various external proton concentrations and to return to a similar Em value at each external pH.

Since there was no effect of external pH on the Em of maize cells, and since at least one earlier study (48) reported a slight hyperpolarization of the Em of oat cells at an external pH of 5.5 or lower, the low temperature studies of both tissues were carried out at pH 5.5. This should allow the response of Em to low temperature to be observed under optimal conditions for the maintenance of both active and passive components of the Em.

The Effect of Temperature on the Response of Em to Increased Illumination

In this study, cells which had established a resting potential at 0.2 W/m^2 were suddenly given increased illumination (3 W/m^2), and the immediate response of Em to this change was monitored. The typical response was qualitatively similar in the two species (Fig. 3). In either species the first response to the increase in light intensity was a partial depolarization of the membrane, followed by a gradual repolarization and hyperpolarization, which is similar to the response

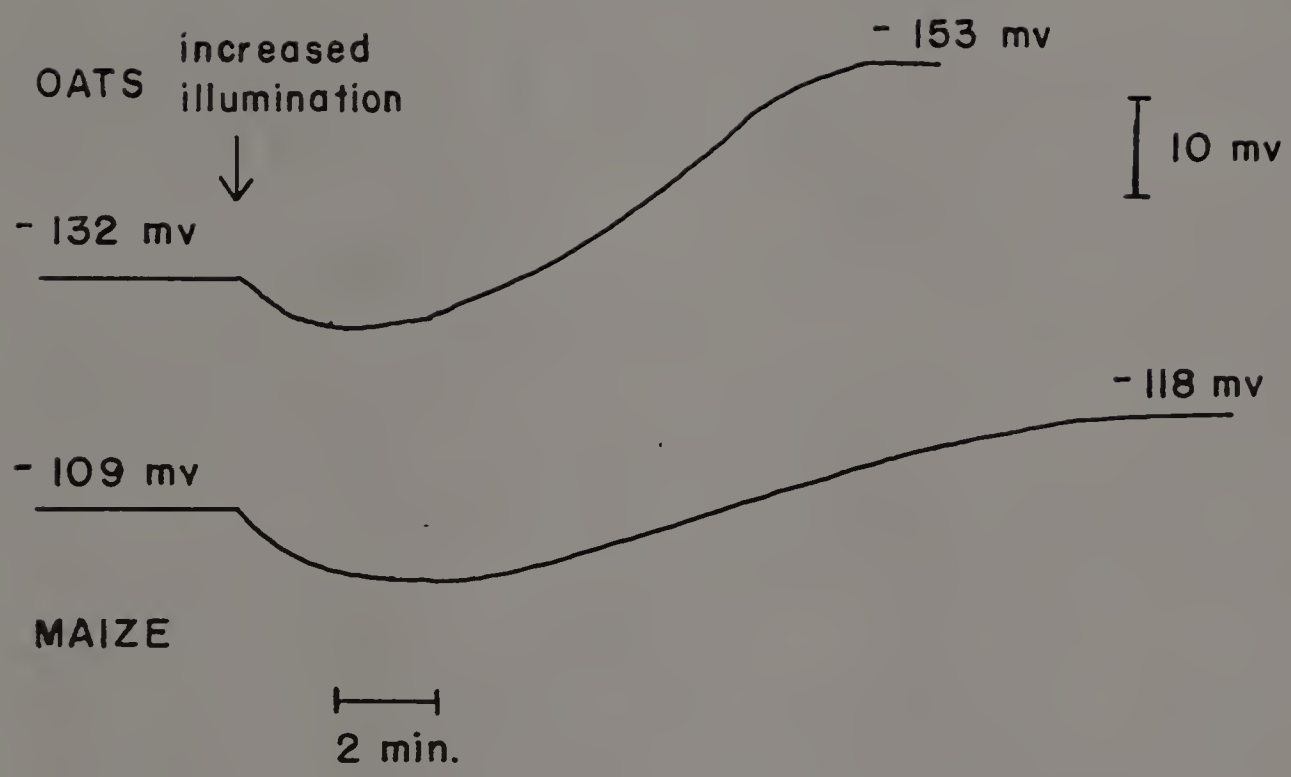

Fig. 3. The response of membrane potential to suddenly increased illumination.

seen by other workers in these and other plants (71). Since monitoring of the Em was stopped when hyperpolarization was completed, and since it is possible that further Em oscillations followed this hyperpolarization, no values for resting potential at the higher light intensity were obtained. In oats, it was clear from a few extended observations that the hyperpolarized Em was part of an oscillation which appeared to damp off slowly. It seemed from observations of the first few oscillations that the final resting potential which would form under increased illumination would fall somewhere between the resting potential at the lower light intensity and the hyperpolarized value, but this was not experimentally verified. Because of the slower overall response in maize the Em was not monitored in any cell long enough to determine the nature of the oscillations past the first hyperpolarization.

As a general comparison between the two species, the membrane potential was usually more negative in oats than in maize, particularly at the higher temperatures and under higher intensity light. The response of the Em to increased illumination was qualitatively similar in the two species at all temperatures used, although there were pronounced quantitative differences (Fig. 4 and 5).

In order to quantify the results from this study for comparison between species and among temperatures, a number of parameters were chosen for analysis. The parameters used were A) the resting potential at the low light intensity, B) the magnitude of the initial depolarization following the increase in light intensity, C) the peak Em

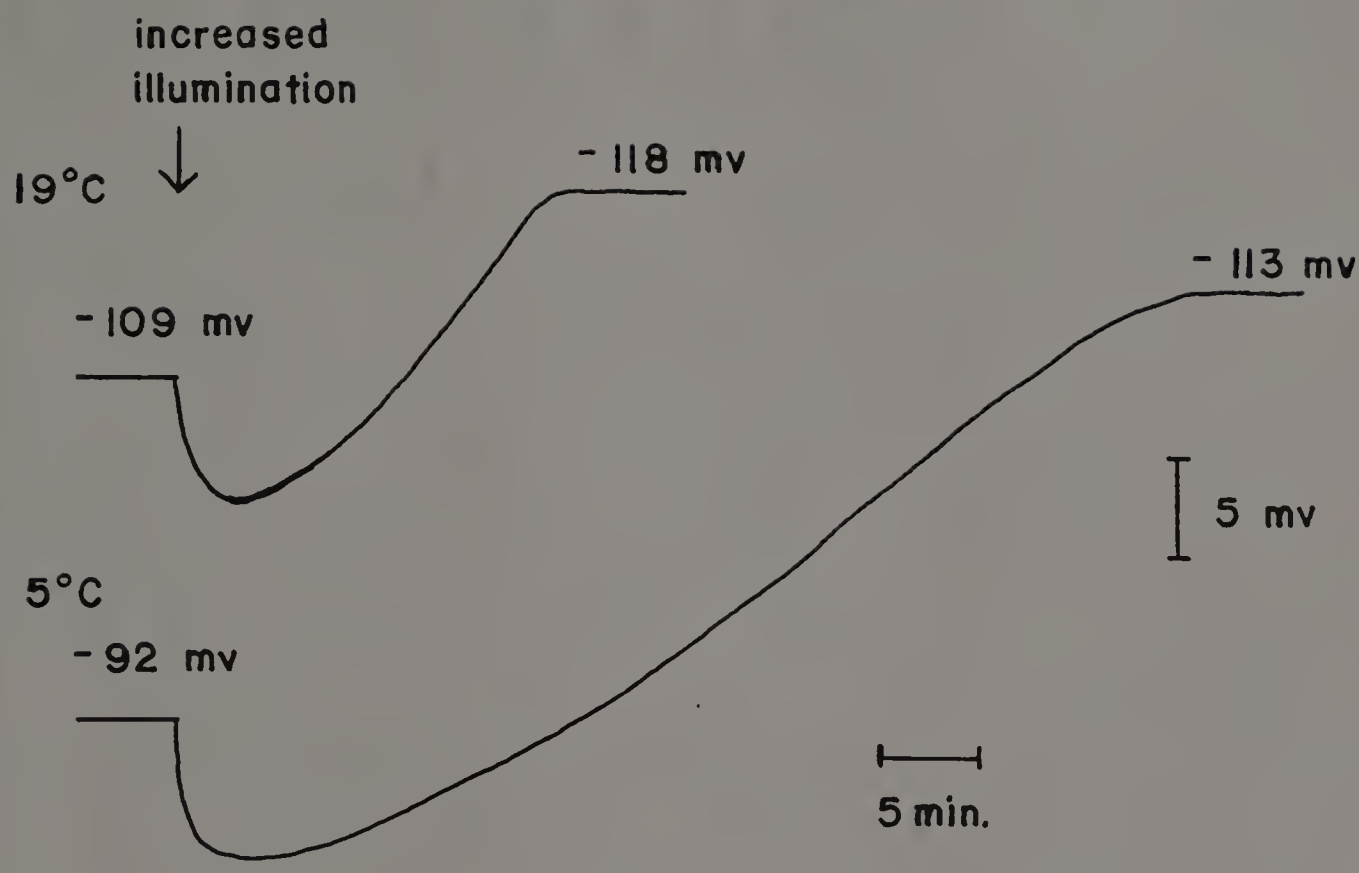

Fig. 5. The effect of temperature on the response of membrane potential to increased illumination in maize.

value, or most hyperpolarized Em, during the first oscillation following the increase in intensity, D) the amplitude of the first oscillation (C - B), E) the time required for completion of the first hyperpolarization, and F) the rate of hyperpolarization during the first oscillation (Fig. 6).

The Effect of Temperature on the Resting Potential at Low Light Intensity

At a light intensity of 0.2 W/m^2, there was a highly significant effect of temperature on the resting potential in both maize and oat cells (Fig.7). There was a 28 mv, or 26%, depolarization of the resting potential of maize cells at 5 C as compared to that at 19 C. In oat cells the same comparison showed a 32 mv, or 24%, depolarization. Although the depolarization with decreasing temperature does not follow a smooth trend in either species, the results in Table 3 show that, contrary to expectations, at most temperatures the depolarization is actually greater in the chilling resistant oat cells than in the chilling sensitive maize cells.

The Effect of Temperature on the Magnitude of the Depolarization following Increased Illumination

Temperature had a highly significant effect on the magnitude of the depolarization following suddenly increased illumination in both maize

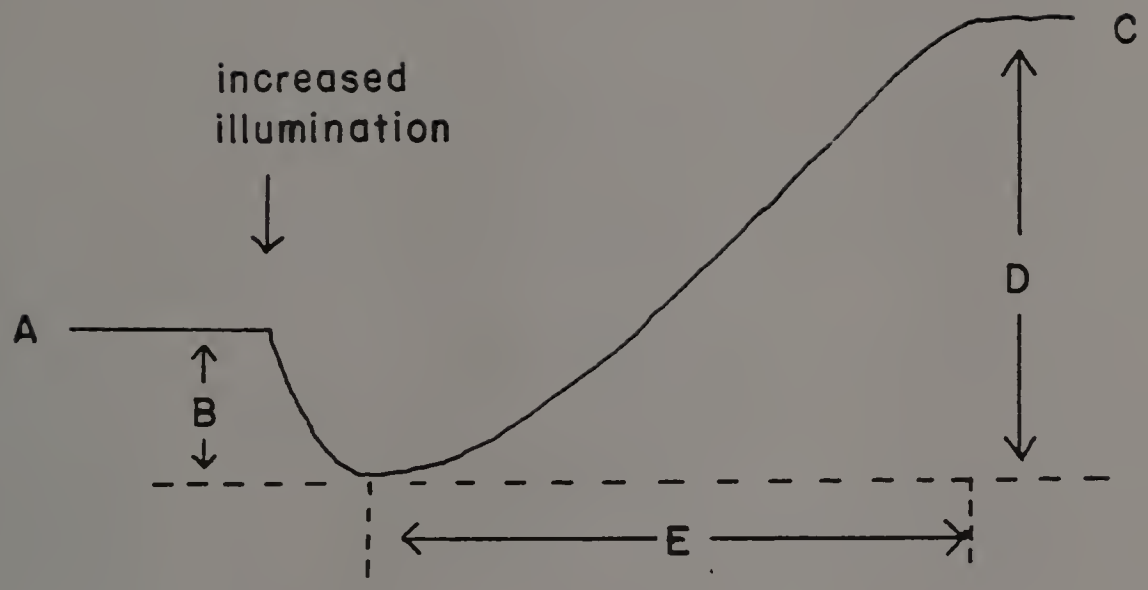

Fig. 6. Analysis of the response of membrane potential to suddenly increased illumination.

A. The resting potential at low light intensity.

B. The magnitude of the depolarization following increased illumination.

C. The peak Em following increased illumination.

D. The Amplitude of the first oscillation following increased illumination.

E. The time required for completion of the first oscillation following increased illumination.

F. The rate of hyperpolarization during the first oscillation following increased illumination (F=D/E).

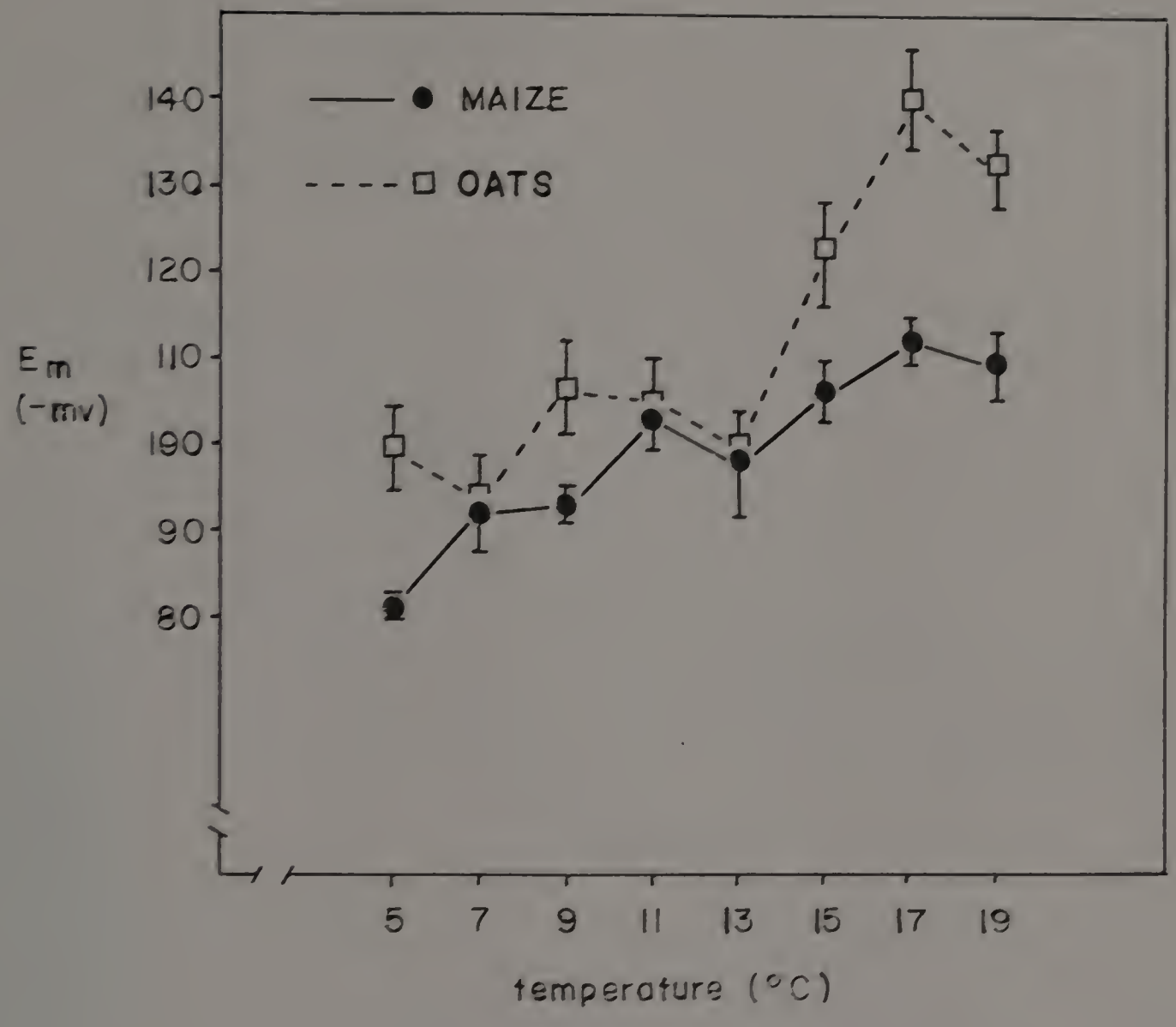

Fig. 7. The effect of temperature on the resting potential at a low light intensity.

TABLE 3. The Response of Membrane Potential to Low Temperatures. The magnitude of the depolarization of the membrane potential caused by low temperatures is expressed at each temperature as a percentage of the total membrane potential at 19 C.

Temperature (C)	Maize	Oats
19	0	0
17	-	-
15	3	8
13	10	24
11	6	20
9	15	19
7	16	29
5	26	24

and oats (Fig. 8). In both species the magnitude was least at the highest temperatures (17 and 19 C) and lowest temperatures (5 and 7 C) and greatest at an intermediate temperature (15 C in maize and 13 C in oats).

The Effect of Temperature on the Peak Em Value following Increased Illumination

There was a highly significant effect of temperature on the peak membrane potential value which was achieved following increased illumination of oat cells (Fig. 9). The same was true in maize only if the value at 5 C was considered. Among the other temperatures studied there was a significant effect only at the 0.05 level of probability, and this effect was due entirely to the low value obtained at 15 C. The mean of the values obtained at temperatures from 7 - 19 C was - 115 mv, considerably higher than the -90 mv value at 5 C.

The values in oat cells fall into two distinct groups; those occurring below 11 C with a mean of - 130 mv, and those occurring above 11 C with a mean of - 152 mv. The individual values found at 11 C also fall into two groups; one with a mean of - 129 mv, and the other with a mean of - 153 mv (Table 4). A t-test of the two groups at 11 C shows that they are significantly different from one another. The value shown as the overall mean at 11 C is therefore misleading, and is actually an average value from two distinct populations.

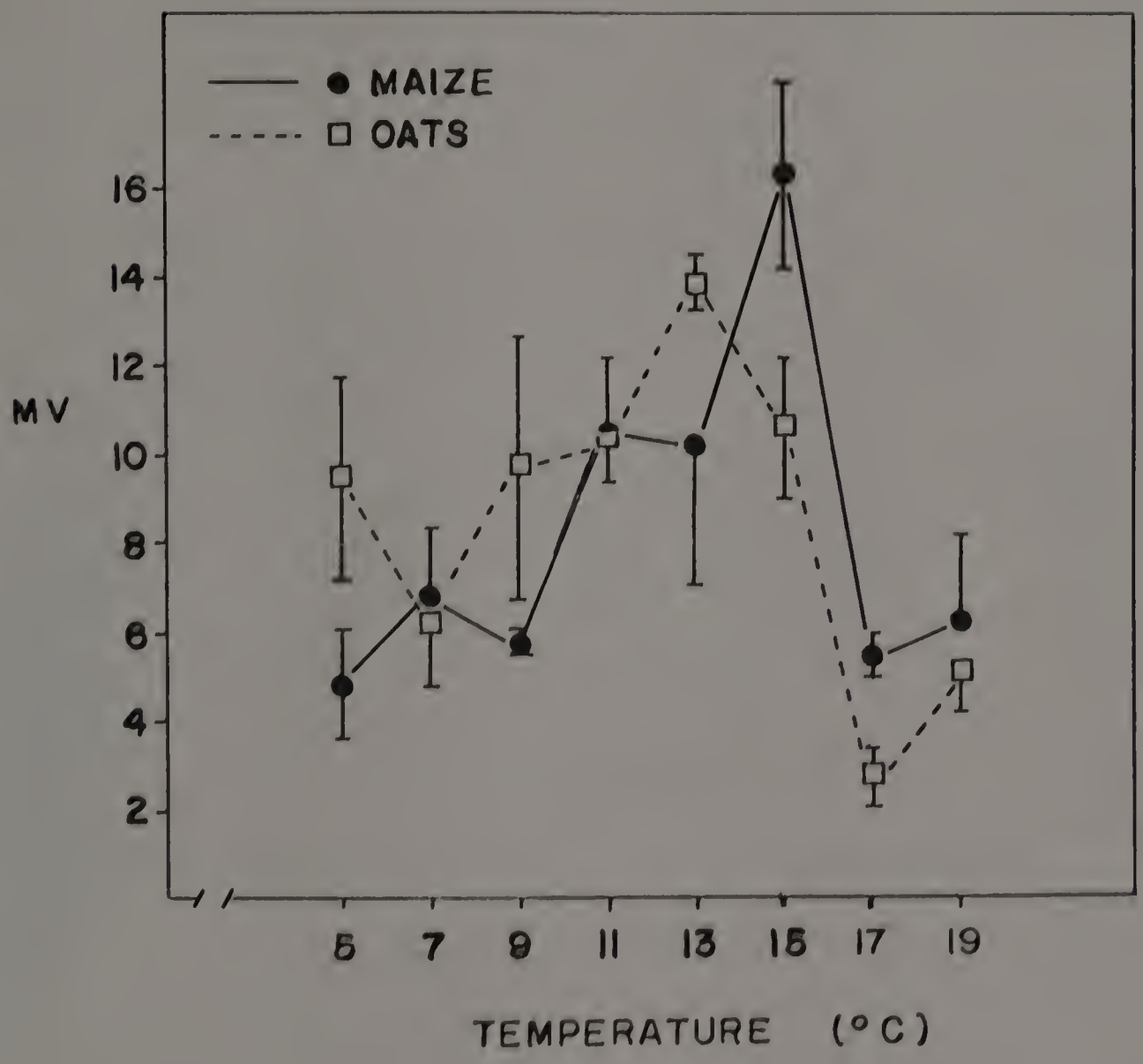

Fig. 8. The effect of temperature on the magnitude of the depolarization following increased illumination.

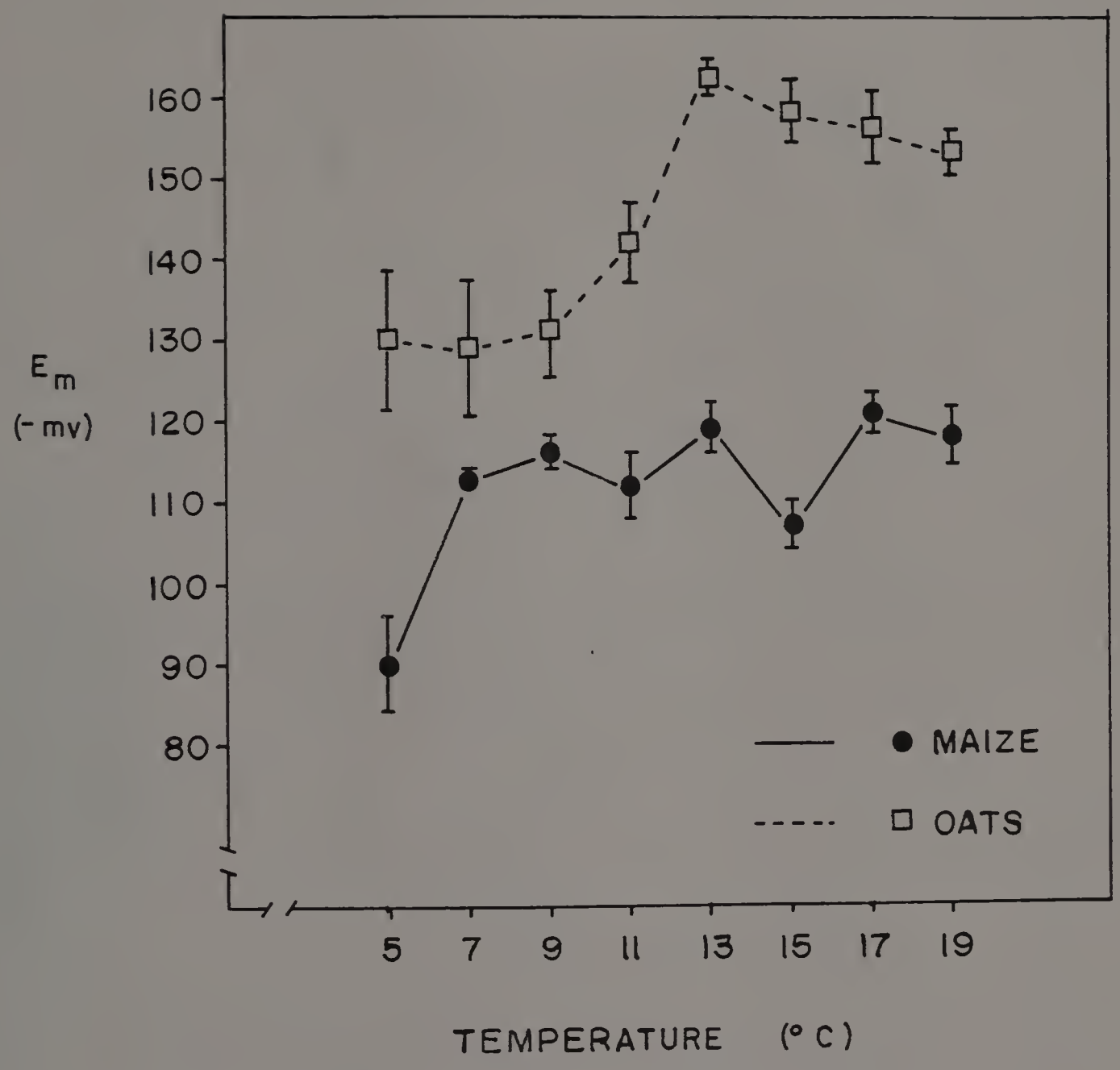

Fig. 9. The effect of temperature on the peak Em following increased illumination.

TABLE 4. The Effect of Temperature on the Peak Em following Sudden Illumination

5 C	7 C	9 C	11 C	13 C	15 C	17 C	19 C	
136	160	115	128	167	170	156	157	
105	132	126	130	162	156	164	156	
144	110	136	128	160	156	148	158	
135	126	138		164	146		148	
	118	152	147	154	161		144	
		120	159	162				
			152					
			152					
130	129	131	142	162	158	156	153	MEANS

The Effect of Temperature on the Amplitude of the Em Oscillation following Increased Illumination

The amplitude of the first oscillation of the Em following a sudden increase of illumination is a function of the magnitude of the initial depolarization and the peak Em value which is attained during hyperpolarization. This value, along with the rate of Em change during hyperpolarization, determines the amount of time required for completion of the first oscillation, and presumably will also affect the amount of time which elapses before a resting potential is established at the higher light intensity.

Temperature had a highly significant affect on the amplitude of the Em oscillation in both species. In oat cells, the amplitude was least at the highest temperatures (17 C and 19 C); fairly low at the lowest temperatures (5 ,7 , and 9 C); and was greatest at 13 C (Fig. 10). In maize cells, the amplitude was again least at the highest (17 and 19 C) and lowest (5 C) temperatures, and greatest at 13 C; however in this case the amplitude remained high between 7 and 13 C.

The Effect of Temperature on the Time Required for Completion of the First Hyperpolarization

In oats, the response of this parameter was significant only at the 0.05 level of probability, and shows no clear trend. In constrast, the response in maize is highly significant and does exhibit some

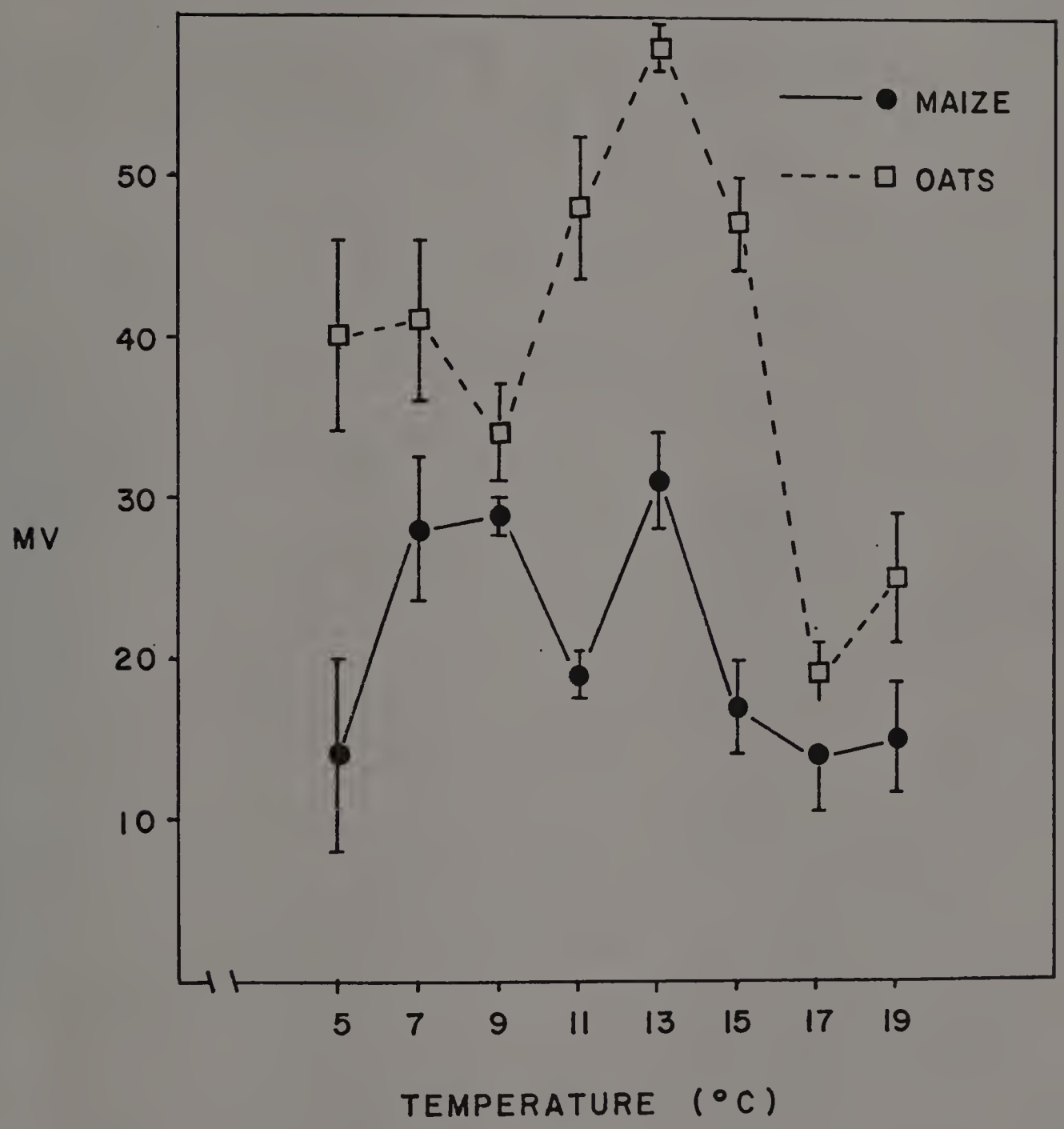

Fig. 10. The effect of temperature on the amplitude of the first oscillation following increased illumination.

interesting characteristics (Fig. 11). A statistical analysis confirms what can be readily observed in Fig. 11; i.e. that the values at 5 - 7 C are not significantly different from one another, that the values at 11 - 19 C are not significantly different from one another, but that there is a highly significant difference between the two groups.

The Effect of Temperature on the Rate of Hyperpolarization following Increased Illumination

An average rate of hyperpolarization was calculated for the entire hyperpolarization process by dividing the amplitude of the first oscillation by the time, in minutes, required for completion of the hyperpolarization (D/E). The response of this parameter to temperature was similar in the two species. The rate of hyperpolarization was greatest at 13 C and decreased as temperature either increased or decreased. In both species, an analysis of variance showed that the effect of temperature on the rate of hyperpolarization was highly significant (Fig. 12).

The Effect of Temperature on Net Proton Extrusion from Leaf Segments

At 19 C in the dark there was no net proton extrusion from oat leaf segments over a five hour period following transfer of the segments from a medium at pH 7.0 and a KCl concentration of 1 mM to a medium at pH 6.5

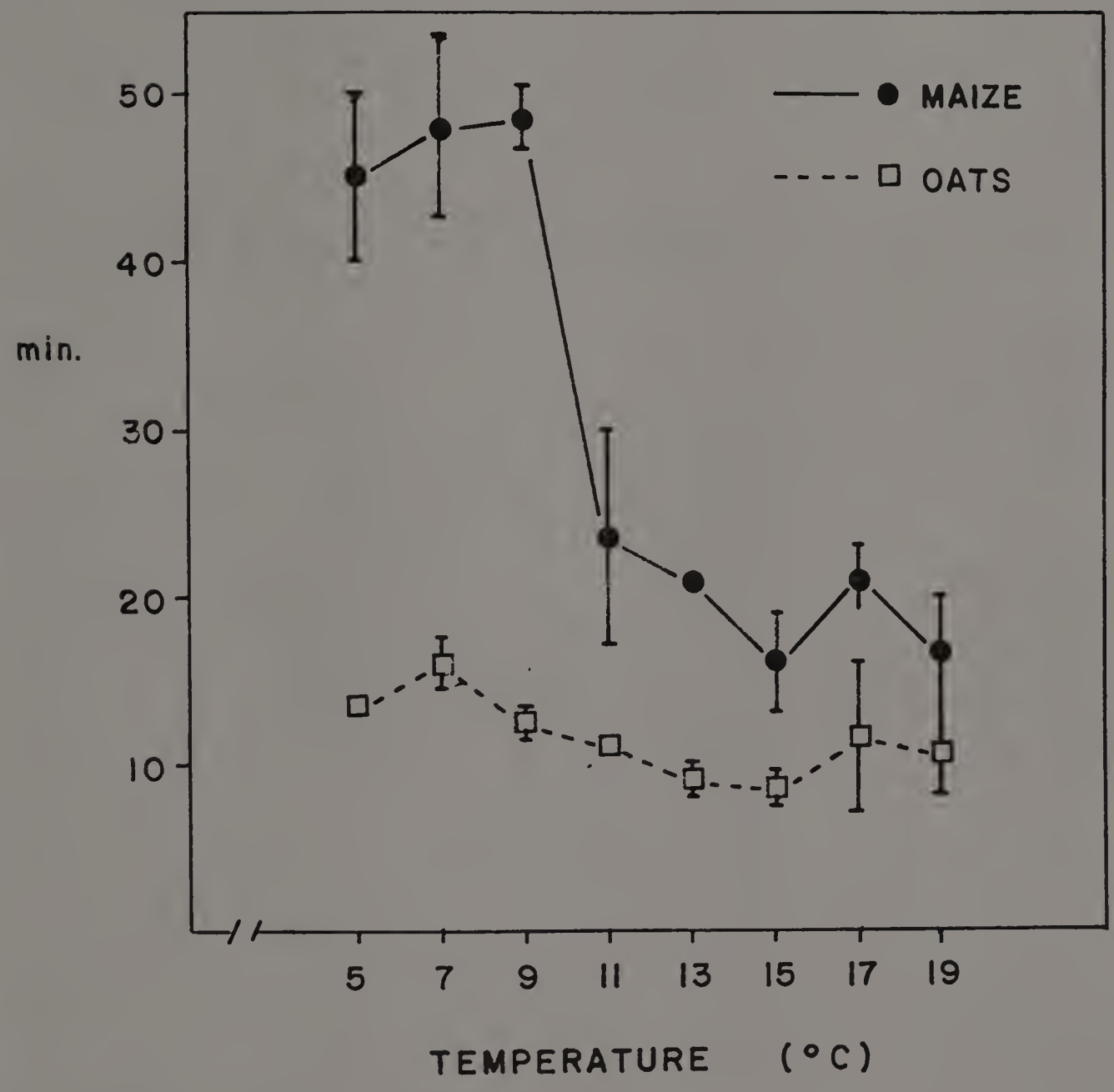

Fig. 11. The effect of temperature on the time required for completion of the first oscillation following increased illumination.

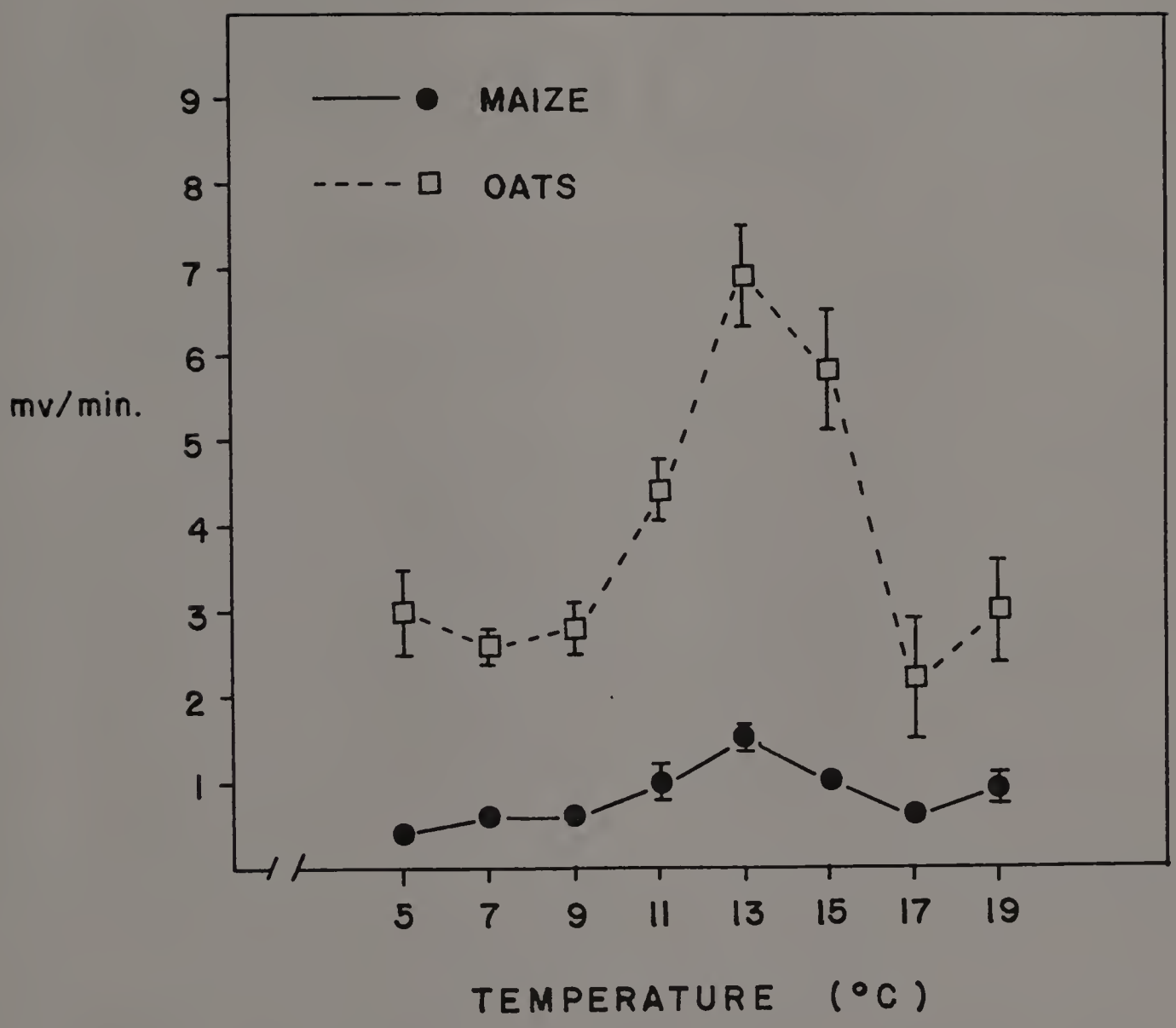

Fig. 12. The effect of temperature on the rate of hyperpolarization during the first oscillation following increased illumination.

and a KCl concentration of 10 mM. Under the same conditions a slight net extrusion of protons was observed in maize. In both species an increase in proton extrusion was observed at 7 C in the dark, although the rate of extrusion was still low (Fig. 13).

Light caused a stimulation of proton extrusion in both species at 19 C and in oats at 7 C, but not in maize at 7 C. Even in oats this light effect was greater at 19 C than at 7 C, so that the greatest rates or extrusion were seen at 19 C in the light (Fig.14). Table 5 shows that in oats the proton extrusion at 7 C is consistently about two-thirds of that at 19 C. In maize, proton extrusion at the lower temperature is initially only about one-fifth of that at 19 C, but within five hours the net extrusion has also reached about two-thirds of that at 19 C.

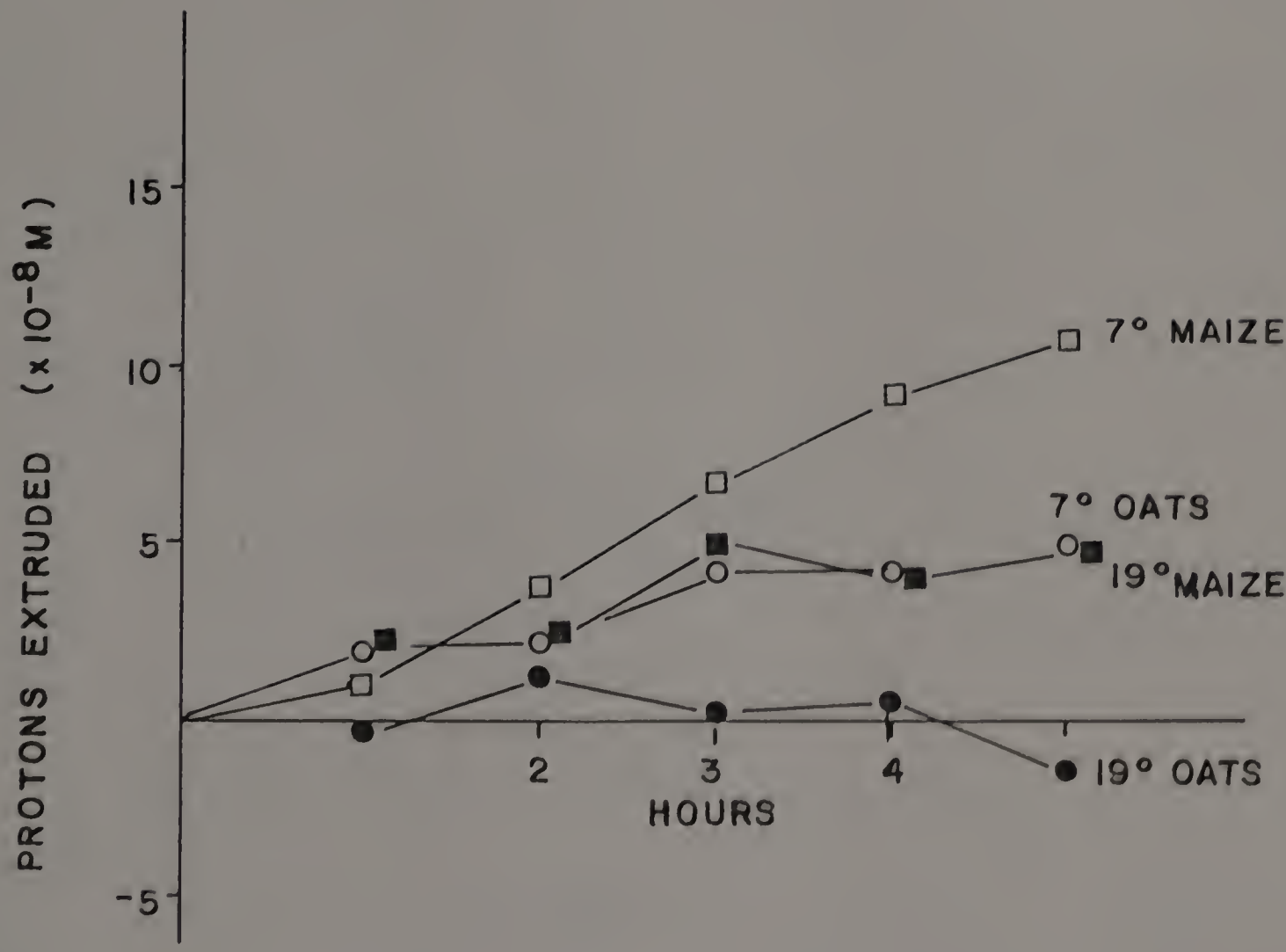

Fig. 13. The effect of temperature on proton extrusion from leaf segments in the dark.

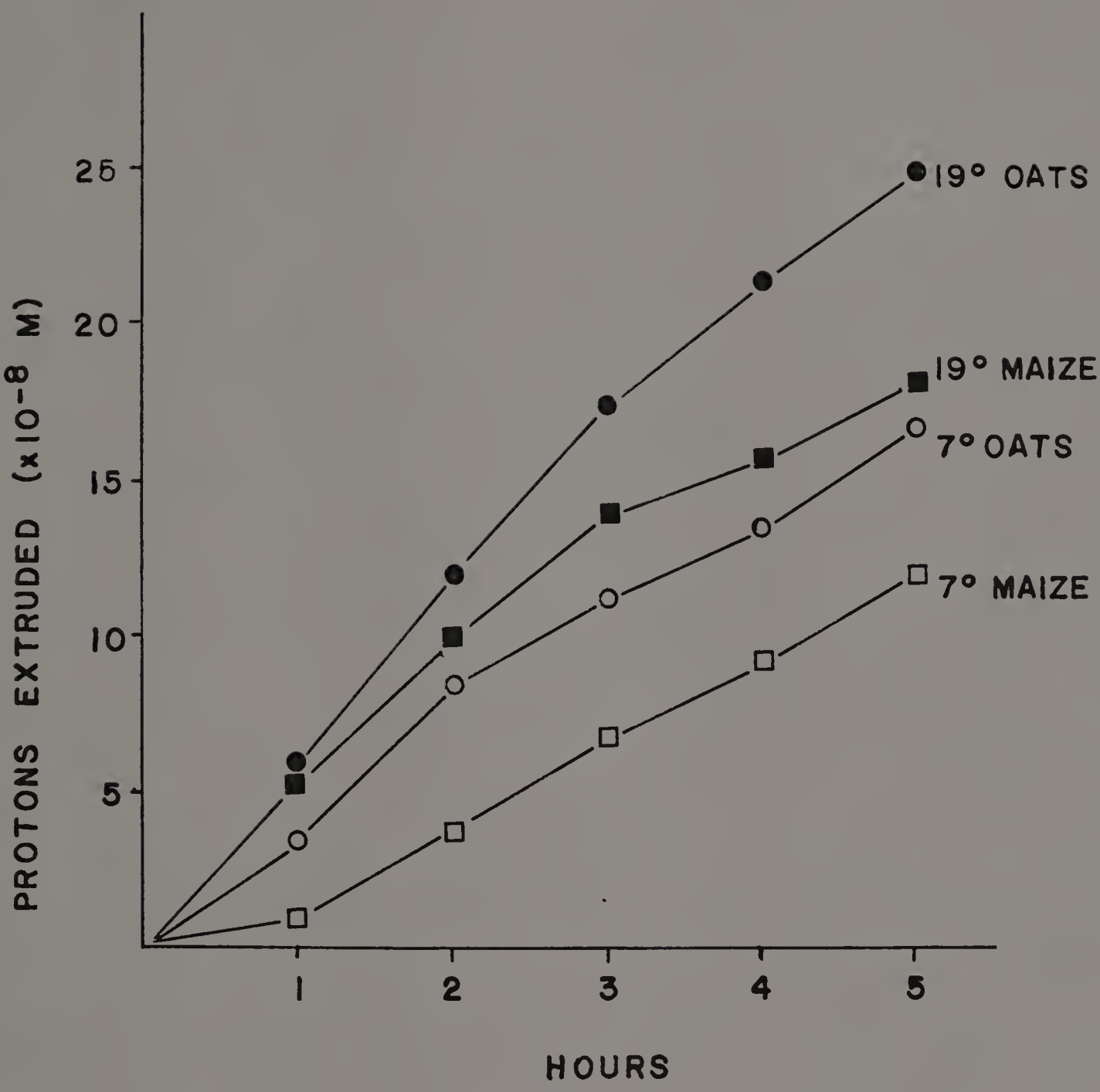

Fig. 14. The effect of temperature on proton extrusion from leaf segments in the light.

TABLE 5. The Effect of Temperature on Proton Extrusion from Leaf Segments in the Light

hours / species	1	2	3	4	5
OATS	58*	71	64	62	67
MAIZE	19	38	48	59	66

* values are the net proton extrusion at 7 C, as a percentage of that at 19 C, and are means of two duplicated experiments.

C H A P T E R V

DISCUSSION AND CONCLUSIONS

Regulation of the membrane potential is clearly a complex process involving an interaction between permeability properties of the membrane, availability of metabolic energy, and characteristics of the electrogenic pump(s). Chilling injury is also complex, and may involve several sites of low temperature interference with physiological functions. Therefore, it is difficult to pinpoint how low temperatures affect membrane potential and how these effects are related to chilling sensitivity, if at all.

A theoretical explanation of the processes involved in producing and regulating the membrane potential, using irreversible thermodynamics, was developed by Rapoport (109) and applied to plant cells by Spanswick (115,117,119). According to their system, the components involved in the maintenance of Em can be treated as as electrical circuit, as seen in Fig. 15, in which the electrogenic pump is represented as a constant voltage source. The concept of the pump as a constant voltage source has been experimentally verified in some algae but not in higher plants. Rapoport's analysis was for a $Na^+ - K^+$ pump, and Spanswick applied the same system to a H^+ pump, although it can also be used for pumps transporting other ions.

In this analysis, the free energy change for active transport of H^+ is expressed as: $$\Delta F_r = \Delta\bar{\mu}_p - v_H \Delta\bar{\mu}_w$$

where $\Delta\bar{\mu}_p$= the free energy change for the non-transported components of

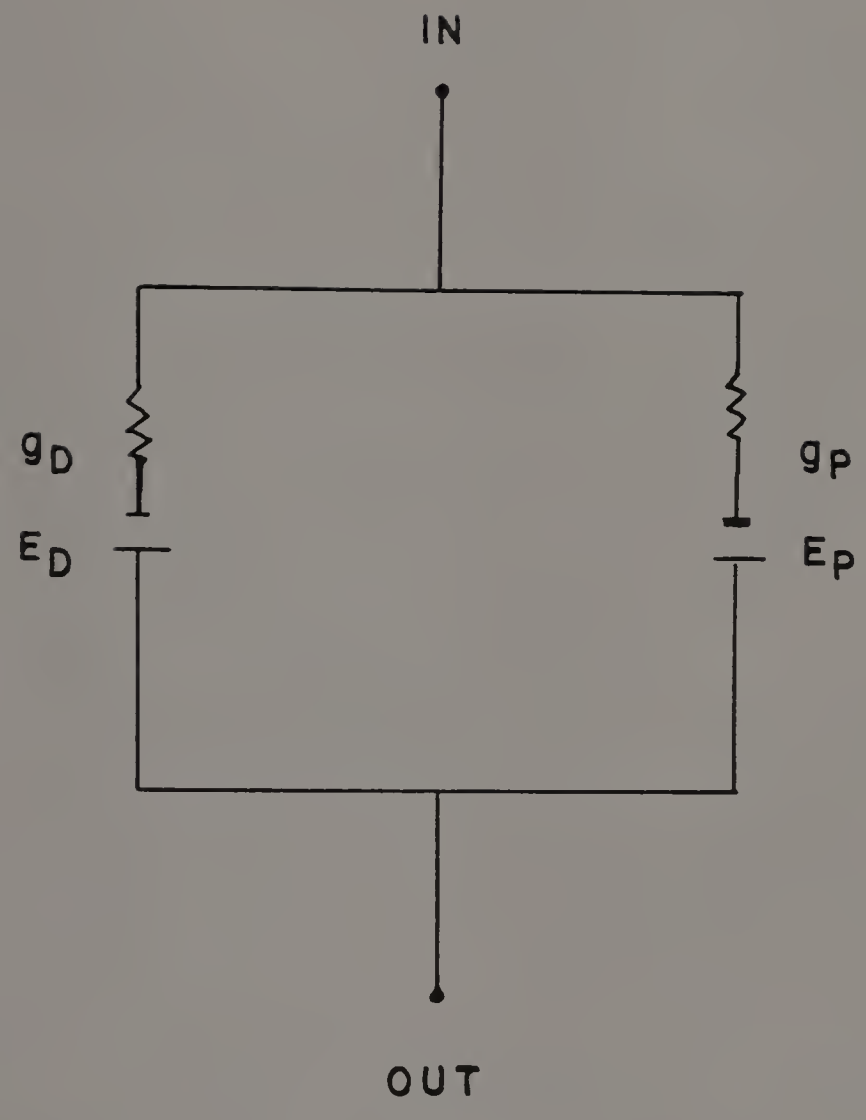

Fig. 15. Circuit analysis of the membrane potential. E_D and E_P are the components of the membrane potential maintained by diffusion and the pump respectively; g_D and g_P represent the conductance of the membrane via diffusion and the pump respectively.

the reaction, v_H= a stoichiometric coefficient, and $\Delta\bar{\mu}_H$= the electrochemical potential difference across the membrane for H^+. The rate of the chemical reaction is expressed as: $J_r = L_{rr}(-F_r)$, where L_{rr}= a thermodynamic conductance coefficient.

When $\Delta\bar{\mu}_P = v_H\Delta\bar{\mu}_H$, the chemical reaction is halted; therefore $J_r = 0$ and $F_r = 0$. This can be expressed as

$$\Delta\bar{\mu}_P = V_H\left(RT \ln \frac{H_i^+}{H_o^+} + FE\right)$$

and the equation can then be used to identify the Em at which the reaction has been halted:

$$E_m = \frac{\Delta\bar{\mu}_P}{Fv_H} - \frac{RT}{F} \ln \frac{H_i^+}{H_o^+}$$

Changes in the electrogenic component of Em with illumination or with changing temperature can be attributed to changes in energy availability or in efficiency of the pump mechanism, each of which would affect $\Delta\bar{\mu}_P$; or to changes in v_H; as well as to permeability changes which would affect the ratio of H_i^+ to H_o^+ (where H_i^+=the internal H^+ concentration and H_o^+=the external H^+ concentration). The transient depolarization seen upon illumination could still be accounted for by temporary changes in H^+. Use of these equations does not preclude the existence of as yet unidentified regulatory processes, but does explain how the membrane potential is regulated under the simplest of circumstances.

The results of the present study are discussed below as they relate to this simple theoretical base and this analysis is used to explain the observed behavior. The discussion also focuses on pumps at the

plasmalemma, but with the awareness that the electrophysiological techniques used make it impossible to differentiate between events occurring at the plasmalemma versus events occurring at the tonoplast, or interactions between the membrane and the microelectrode.

The Effect of Temperature on the Resting Potential at a Low Light Intensity

The resting potential of maize and oat leaf cells at 19 C and low light intensity is clearly more negative than the diffusion potential as reported by Jennings and Tattar (55). In their work, the addition of 1 mM sodium azide at 21 C brought the membrane potential to about - 60 mv in maize and about - 70 mv in oats. In comparison to these values, there appears to be an electrogenic component of the Em even at 5 C (Fig. 7). Nelles and Laske (90) showed that the diffusion potential of corn coleoptile cells became less negative at lower temperatures, suggesting that in this study the electrogenic component at 5 C may be even greater than is apparent by comparison to the diffusion potential at 21 C. The magnitude of the change in deffusion potential at low temperatures seen by Nelles and Laske was less than the change seen in this study, indicating that the decreased negativity seen here can be attributed, at least in part, to changes in the active component of the Em.

The gradual change in Em values with temperature is most likely related to changes in the level of energy available to drive the

electrogenic pump(s), thereby causing a change in $\tilde{\mu}_p$. However a more direct low temperature effect on the transport mechanism itself, presumably an ATPase, is also possible. Because of the gradual change in Em with temperature, any effect of temperature on the ATPase is probably an effect on the rate of its activity (again showing up as a change in $\Delta\tilde{\mu}_p$) rather than a change in the stoichiometry of the reaction (which is a change in v_H).

In neither species was there a sudden drop in the Em at a specific temperature which would be indicative of a membrane phase transition. Also, as was pointed out earlier, the decreased negativity of the Em at low temperatures was more pronounced in oats than in maize. The similarity of the response in the two species makes it unlikely that this response is related to chilling injury or resistance.

The Effect of Temperature on the Magnitude of Depolarization following Increased Illumination

The depolarization of the Em which is the initial response to sudden illumination in both maize and oats apparently depends on the interaction of various factors controlling Em. Thus the effect of temperature on this response is difficult to interpret clearly.

According to present theories regarding the cause of this depolarization, it is a reflection of either sudden changes in the internal H^+ concentration, related in some way to photosynthetic light reactions (28,71,93), or changes in membrane permeability, again

triggered by some product of photosynthesis (8,137). In Nitella there is evidence that there may be three parallel reaction pathways between the chloroplasts and the membrane potential (45,81). The repolarization which follows is stimulated by the drop in the Em and possibly by the additional energy supplied by photophosphorylation. Therefore the magnitude of the depolarization depends on an interaction between the strength of the depolarizing signal and the lag time before electrogenic activity can counteract the response.

In both species studied the magnitude of the depolarization was least at the highest temperatures, greatest at intermediate temperatures, and low again at the lowest temperatures (Fig. 8). The photosynthetic light reaction products which are theorized to trigger the initial depolarization are likely to accumulate quickest and to the greatest extent at the highest temperatures, causing the "depolarizing signal" to be strongest at these temperatures. Therefore, it seems likely that the low degree of depolarization at these temperatures reflects a rapid response of the electrogenic system. The increased magnitude of depolarization at intermediate temperatures could be caused by a differential effect of temperature on the two responses, i.e., the light reaction products which act as the "depolarizing signal" may be only slightly affected at these temperatures, while the reaction of the electrogenic system is more strongly inhibited. Work on the low temperature effects on photosynthetic light reactions, often show Arrhenius plot breaks occurring between 10 C and 15 C, with little temperature effect above these breaks (25,91,113), which is consistent

with this theory. The decreased magnitude of depolarization at the lowest temperatures may indicate that at these temperatures the strength of the depolarizing signal has also been inhibited strongly, and that the two responses are again more closely balanced. A graphic representation of how differential effects of low temperature on light reactions of photosynthesis and on pump activity could interact to give the observed results is provided in Fig. 16. Although this is clearly an oversimplification of the process, it shows how those reactions causing depolarization and those causing repolarization can change separately, thereby changing the balance between the two and changing the overall magnitude of the depolarization.

The response of this parameter to temperature is qualitatively and quantitatively similar in maize and oats, again indicating no significance of this parameter to the difference in chilling sensitivity of the two species. The greatest depolarization occurred at 15 C in maize and 13 C in oats, which might mean that the low temperature inhibition of electrogenic activity occurs at a higher temperature in maize. However the difference is not great enough to warrant any correlation between this response and chilling sensitivity.

The Effect of Temperature on the Peak Em following Increased Illumination

The peak Em, or most hyperpolarized Em, following increased illumination was the parameter giving the most interesting results in

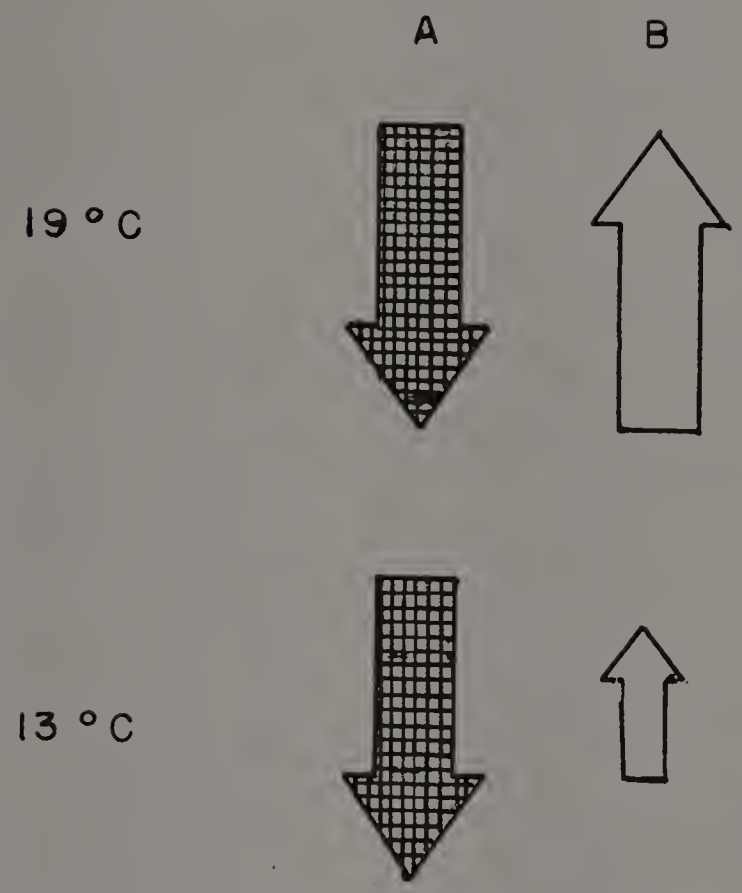

Fig. 16. Regulation of membrane potential depolarization upon increased illumination. A. The magnitude of the "depolarizing signal", B. The activity of the electrogenic pump. If production of the depolarizing signal is less affected by temperature than the activity of the pump, an imbalance between the two could arise at intermediate temperatures which would lead to greater depolarization at these temperatures.

terms of its response to temperature and the difference in the temperature response between oats and maize.

Despite the low value observed at 15 C (Fig. 9), it appears that maize cells reach the same peak value regardless of temperature , in the range from 7 - 19 C. This result indicates that the electrogenic portion of the total Em is greater at the lower temperatures, which would not be expected based on changes in the activation energy of the elctrogenic pump or in the cytoplasmic energy supply. Nelles and Laske (90) have explained similar results by suggesting that at low temperatures an additional electrogenic pump is activated, possibly through changes in the lipid composition of the plasma membrane induced by low temperatures.

An explanation of these results using the circuit analysis is possible, and does not require the presence of any other electrogenic pump. The constancy of the peak Em value over temperature suggests that, under conditions in which the energy supply is not limiting, the membrane potential is regulated by voltage alone, as proposed above. For maize, the Em at which $\Delta\bar{\mu}_{\rho} = v_H \Delta\bar{\mu}_H$ appears to be ~ - 115 mv. If the pump is voltage regulated at high light intensity, then the results of this study indicate that the energy supply is rate-limiting at lower temperatures in the dark (or at low light intensities). The sudden drop in the peak Em at 5 C under high intensity light could be caused by a low temperature inhibition of photophosphorylation, and thus a drastic drop in energy availability, or by a shut-down of the electrogenic pump at this temperature.

Support for this explanation is derived from the temperature effect on the rate of hyperpolarization (Fig. 12). If the constancy of the peak Em over temperature is a result of increased activity of the electrogenic systems, as suggested by Nelles and Laske, then the rate of hyperpolarization at low temperatures should be the same or greater than the rate at 19 C. The data obtained in this study can not be reconciled with such an approach, which treats the electrogenic pump as a constant current source, but can be explained when the electrogenic pump is treated as a constant voltage source. Using this theory, the low rate of hyperpolarization at high temperatures can be explained by the fact that the Em has remained close to its most hyperpolarized value, causing the rate of the reaction to slow down as it reaches thermodynamic equilibrium. At intermediate temperatures the rate of the reaction is not limited by the proximity of the Em to its equilibrium value, so the rate increases. As the temperature is decreased further the availability of ATP to drive the reaction may be decreased by low temperature effects on photophosphorylation and respiration, and the activity of the pump may be directly affected. These factors could be expected to decrease the rate of hyperpolarization without having any affect on the peak Em value which is reached.

The results seen in oats differ from those in maize, but can still be explained on the same basis. The peak Em in oats is different at temperatures below 11 C than it is at temperatures above 11 C (Fig. 9). A change in the Em at which the pump is turned off is possible if the stoichiometric coefficient, v_H, increases. An increase in the

stoichiometric coefficient would also result in an increased efficiency of the electrogenic pump. Changes in the stoichiometry of pump ATPases have been observed in animal cells and mitochondria, and such a change has been postulated to occur in Neurospora under conditions of chromic energy restriction (139). Such a change could be caused by a change in the conformation of the ATPase which acts as the pump, which in turn coud be caused by a phase transition of the plasmalemma lipids at a temperature near 11 C.

The abrupt change in peak Em at 11 C is as would be predicted from a phase transition, and is difficult to explain in terms of effects on the rate of a chemical reaction. The occasional highly negative peak Em seen in oat cells at temperatures below 11 C is probably associated with a cell which for some reason has not yet undergone phase transition (Table 5). The absence of this response in maize does not necessarily indicate the absence of a plasmalemma phase transition, but may be related instead to differences in the ATPases of the two species.

The significance of this difference between maize and oats in the response of their electrogenic pump to low temperature cannot be confirmed by the results of this study, but one possibility is suggested. Workers have suggested previously that CO_2 fixation may compete with the electrogenic pumps for available energy (68,115). This was suggested by Spanswick following the observation that light-stimulated hyperpolarization of the Em of Nitella translucens was inhibited by the presence of CO_2 in the external medium; and by Luttge, et al. when they observed that light caused an inhibition of anion

uptake under some conditions and a stimulation under others, which they believed was related to the nutritional status of the tissue.

If it is true that CO_2 fixation and electrogenic pumps compete for available ATP it is possible that the maintenance of the membrane potential at low temperatures causes a drain on the energy supply, which in turn limits the synthesis of carbohydrates. The fact that in oats the electrogenic pump is turned off at a less negative Em at lower temperatures may allow for the conservation of energy which can then be used for CO_2 fixation and sustained growth. The Em maintained at low temperatures may partially limit transport of carbohydrates, amino acids, and ions as compared to that maintained at higher temperatures. However, it may be more advantageous for the plants to sustain CO_2 fixation than to maintain optimal intercellular transport rates, under conditions in which energy is limited.

In maize, no change in the peak Em value is seen above 5 C. This would result in a continued drain on the energy supply in these plants which could be responsible for the decrease in growth observed at low temperatures, as well as affecting a number of other energy dependent physiological processes.

The amount of energy required to maintain a particular Em value depends on the energy efficiency of the electrogenic pumps and the permeability characteristics of the membranes. Since these factors are likely to vary among species, the absolute values of the peak Em maintained at low temperatures in the two species can not be used to estimate the extent of the energy drain it causes.

The Effect of Temperature on the Amplitude of the First Oscillation following Increased Illumination

The amplitude of the oscillation which follows increased illumination appears to be limited primarily by the voltage dependence of the electrogenic pump. Although it is also a function of the resting potential at low light intensity and the initial depolarization which occurs upon increasing the light intensity, there does not appear to be any upper limit on the oscillation imposed by any factor other than the attainment of the peak Em value. Therefore analysis of the temperature effects on this parameter provide no additional information as to the regulation of Em or the differences between maize and oats in their response to low temperatures.

The Effect of Temperature on the Time Required for Completion of the First Oscillation

As with some of the parameters discussed above, this parameter is a function of the interaction between the factors which are directly involved in regulating the Em and does not provide any additional information as to how low temperatures affect these factors. However, the results shown in Fig. 11 do provide perspective on the extent of the low temperature effects in maize. At temperatures below 11 C, recovery from the initial effects of increased illumination takes about

2.5 times as long as it does at higher temperatures. In a dynamic situation where the membrane potential is constantly being partially dissipated by transport and other processes, the energy supply of maize cells would be continuously diverted to the electrogenic pump at the expense of other metabolic functions.

The Effect of Temperature on Proton Extrusion from leaf Segments

Although low temperatures were shown to partially inhibit the extrusion of protons from both oats and maize, correlation of these results with effects on electrogenic pumps is impossible. Kelly and Rubinstein (58) found evidence that the apparent light-stimulation of proton extrusion from oat leaves may instead be a stimulation caused by photosynthetic O_2 evolution, which provides O_2 for respiratory energy production. If this is true then the decrease in proton extrusion at low temperatures could be an affect on photosynthetic evolution and respiration, rather than any direct temperature effect.

Conclusions

The results presented above are consistent with the concept of a voltage-regulated electrogenic pump in higher plants, as has been suggested for some algae. Assuming that such a system does exist in higher plants, then the evidence also suggests that:

1) The voltage dependence of the electrogenic pump is temperature dependent in oats but not in maize.

2) In oats, the low temperature induced change in the voltage dependence of the electrogenic pump may act as an energy-conserving mechanism, and enhance CO_2 fixation at low temperatures. Similarly, the absence of this response in maize may limit CO_2 fixation in this species at low temperatures.

LITERATURE CITED

1. Amin, J.V. 1969. Some aspects of respiration and respiration inhibitors in low temperature effects of the cotton plant. Physiol. Plant. 22: 1184-1191.

2. Balke, N.E. and T.K. Hodges. 1979. Effect of diethylstilbestrol on ion fluxes in oat roots. Plant Physiol. 63:42-47.

3. Balke, N.E. and T.K. Hodges. 1979. Inhibition of adenosine triphosphatase activity of the plasma membrane fraction of oat roots by diethylstilbestrol. Plant Physiol. 63:48-52.

4. Balke, N.E. and T.K. Hodges. 1979. Comparison of reductions in adenosine triphosphate content, plasma membrane-associated adenosine triphosphate activity, and potassium absorption in oat roots by diethylstilbestrol. Plant Physiol. 63:53-56.

5. Bates, G.W., M.H.M. Goldsmith, and T.H. Goldsmith. 1981. Electrical properties of thhe tonoplast of oat coleoptile cells. Plant Physiol. 67:S-161.

6. Beffagna, N., S. Cocucci, and E. Marre. 1977. Stimulating effect of fusicoccin on K^+-activated ATPase in plasmalemma preparations from higher plant tissues. Plant Sci. Lett. 8:91-98.

7. Bennett, A., F.M. Dupont, and R.M. Spanswick. 1981. Measurement of ATP-dependent proton transport in membrane vesicles from corn roots. Plant Physiol. 67:S-9.

8. Bentrup, F.W., H. J. Gratz, and H. Unbehauen. 1973. The membrane potential of Vallisneria leaf cells: Evidence for light-dependent proton permeability changes. In, Ion Transport in Plants, W.P. Anderson, ed., Academic Press, New york/London. pp. 171-187.

9. Blinks, L.R. 1935. Protoplasmic potentials in Halicystis IV. Vacuolar perfusion with artificial sap and sea water. J. Gen. Physiol. 18:409.

10. Bravo-F., P. and E.G. Uribe. 1981. An analysis of the relationship between respiration and the transmembrane potential in corn roots. Plant Physiol. 67:808-814.

11. Bravo-F., P. and E.G. Uribe. 1981. Temperature dependence of the concentration kinetics of absorption of phosphate and potassium in corn roots. Plant Physiol. 67:815-819.

12. Breidenbach, R.W. and A.J. Waring. 1977. Response to chilling of tomato seedlings and cells in suspension cultures. Plant Physiol. 60:190-192.

13. Brinckmann, E. and U. Luttge. 1974. Lichtabhangige Membranpotentialschwankungen und deren interzellulare Weiterleitung bei panaschierten Photosynthese-Mutanten von Cenothera. Planta 119:47-57.

14. Briskin, D.P. and R.T. Leonard. 1979. Ion transport in isolated protoplasts from tobacco suspension cells. III. Membrane potential. Plant Physiol. 64:959-962.

15. Carey, R.W. and J.A. Berry. 1978. Effects of low temperature on respiration and uptake of rubidium ions by excised barley and corn roots. Plant Physiol. 61:858-860.

16. Cheeseman, J.M. and J.B. Hanson. 1979. Mathematical analysis of the dependence of cell potential on external potassium in corn roots. Plant Physiol. 63:1-4.

17. Cheeseman, J.M. and J.B. Hanson. 1979. Energy-linked potassium influx as related to cell potential in corn roots. Plant Physiol. 64:842-845.

18. Chen, T. and R.M. Spanswick. 1982. The membrane potential of soybean root tip cells. Plant Physiol. 69:S-47.

19. Christiansen, M.N., H.R. Carns, and D.J. Slyter. 1970. Stimulation of solute loss from radicles of Gossypium hirsutum L. by chilling, anaerobiosis, and low pH. Plant Physiol. 46:53-56.

20. Churchill, K.A. and H. Sze. 1982. ATP-generated pH gradient in sealed microsome vesicles from oat roots. Plant Physiol. 69:S- 66.

21. Churchill, K.A. and H. Sze. 1982. Relationship between ATP-generated pH gradient, electrical potential, and ATPase activities in microsomal vesicles from plant tissues. Plant Physiol. 69:S-66.

22. Cocucci, M., A. Ballarin-Denti, and M.T. Marre. 1980. Effects of orthovanadate on H^+ secretion, K^+ uptake, electric potential difference and membrane ATPase activities of higher plant tissues. Plant Sci. Lett. 17:391-400.

23. Contardi, P.J. and R.F. Davis. 1978. Membrane potential in Phaeoceros laevis : Effect of anoxia, external ions, light, and inhibitors. Plant Physiol. 61:164-169.

24. Creencia, R.P. and W.J. Bramlage. 1971. Reversibility of chilling injury to corn seedlings. Plant Physiol. 47:389-392.

25. Critchley, C., R.M. Smillie, and B.D. Patterson. 1978. Effect of temperature on photoreductive activity of chloroplasts from passionfruit species of different chilling sensitivity. Aust. J. Plant Physiol. 5:443-448.

26. Crookston, R.K., J. O'Toole, R. Lee, J.L. Ozbun, and D.H. Wallace. 1974. Photosynthetic depression in beans after exposure to cold for one night. Crop Sci. 14:457-464.

27. Dainty, J. 1962. Ion transport and electrical potentials in plant cells. Ann. Rev. Plant Physiol. 13:379-402.

28. Davis, R.F. 1974. Photoinduced changes in electrical potentials and H activities of the chloroplast, cytoplasm, and vacuole of Phaeoceros laevis. In Membrane Transport in Plants, U. Zimmermann and J. Dainty,eds., Springer, Berlin, pp. 197-201.

29. Denny, P. and D.C. Weeks. 1970. Effects of light and bicarbonate on membrane potential in Potamogeton schweinfurthii (Benn). Ann. Bot. 34:483-496.

30. Drake, B. and K. Raschke. 1974. Prechilling of Xanthium strumarium L. reduces net photosynthesis and, independently, stomatal conductance, while sensitizing the stomata to CO_2. Plant Physiol. 53:808-812.

31. DuPont, F.M., A. B. Bennett, and R.M. Spanswick. 1981. Characterization of ATP-dependent proton transport in membrane vesicles from corn roots. Plant Physiol. 67:S-9.

32. Etherton, B. and B. Rubinstein. 1978. Evidence for amino acid-H^+ co-transport in oat coleoptiles. Plant Physiol. 61:933-937.

33. Felle, H. and W. Bentrup. 1974. Light-dependent changes of membrane potential and conductance in Riccia fluitans. In, Membrane Transport in Plants. U. Zimmerman and J. Dainty, eds. Springer, Berlin,pp. 120-125.

34. Fisher, J.D., D. Hansen, and T.K. Hodges, 1970. Correlation between ion fluxes and ion-stimulated adenosine triphosphatase activity of plant roots. Plant Physiol. 46:812-814.

35. Garber, M.P. 1977. Effect of light and chilling temperatures on chilling-sensitive and chilling-resistant plants. Pretreatment of cucumber and spinach thylakoids in vivo and in vitro. Plant Physiol. 59:981-985.

36. Garrard, L.A. and S.H. West. 1972. Suboptimal temperature and assimilate accumulation in leaves of "Pangola" Digitgrass (Digitaria decumbens Stent.). Crop Sci. 12:621-623.

37. Giaquinta, R.T. 1979. Phloem loading of sucrose. Involvement of membrane ATPase and proton transport. Plant Physiol. 63:744-748.

38. Goldmann, D.E. 1943. Potential, impedance, and rectification in membranes. J. Gen. Physiol. 27:37-60.

39. Gradmann, D. 1970. Einflu von Licht, Temperatur und Au enmedium auf das elektrische Verhalten von Acetabularia crenulata. Planta 93:323-353.

40. Gradmann, D. and F.W. Bentrup. 1970. Light-induced membrane potential changes and rectification in Acetabularia. Naturwissenschaften 57:46-47.

41. Graham, D., D.G. Hockley, and B.D. Patterson. 1979. Temperature effects on phosphoenol pyruvate carboxylase from chilling sensitive and chilling resistant plants. In, Low Temperature Stress in Crop Plants: The Role of the Membrane. J.M. Lyons, D. Graham, J.K. Raison, eds., Academic Pres, New York, pp. 453-461.

42. Gronewald, J.W., J.M. Cheeseman, and J.B. Hanson. 1979. Comparison of the responses of corn root tissue to fusicoccin and washing. Plant Physiol. 63:255-259.

43. Grzesiak, S., S.B. Rood, S. Freyman, and D.J. Major. 1981. Growth of corn seedlings: Effects of night temperature under optimum soil moisture or under drought conditions. Can. J. Plant Sci. 61:871-877.

44. Guinn, G. 1971. Chilling injury in cotton seedlings: Changes in permeability of cotyledons. Crop Sci. 11:101-102.

45. Hansen, U.P. 1978. Do light-induced changes in the membrane potential of Nitella reflect the feed-back regulation of a cytoplasmic parameter? J. Membr. Biol. 41:197-224.

46. Hiatt, A.J. 1968. Electrostatic association and Donnan phenomena as mechanisms of ion accumulation. Plant Physiol. 43:893-901.

47. Higinbotham, N. 1973. Electropotentials of plant cells. Ann. Rev. Plant Physiol. 24:25-46.

48. Higinbotham, N., J.S. Graves, and R.F. Davis. 1970. Evidence for an electrogenic ion transport pump in cells of higher plants. J. Membr. Biol. 3:210-222.

49. Higinbotham, N. and W.P. Anderson. 1974. Electrogenic pumps in higher plant cells. Can J. Bot. 52:1011-1021.

50. Higinbotham, N., B. Etherton, and R.J. Roster. 1964. Effect of external K^+, NH_4^+, Na^+, Ca^{++}, Mg^{++}, and H^+ ions on the cell transmembrane electropotential of Avena coleoptile. Plant Physiol. 39:196-203.

51. Hilliard, J.H., and S.H. West. 1970. Starch accumulation associated with growth reduction at low temperatures in a tropical plant. Science 168:494-496.

52. Hodges, T.K. and R.T. Leonard. 1974. Purification of a plasma membrane-bound adenosine triphosphatase from plant roots. Methods Enzymol. 32B:392-406.

53. Hogg, J., E.J. Williams, and R.J. Johnson. 1968. The temperature dependence of the membrane potential and resistance in Nitella translucens. Biochim. Biophys. Acta 150:640-648.

54. Hope, A.B. and P.A. Aschberger. 1970. Effects of temperature on membrane permeability to ions. Aust. J. Biol. Sci. 23:1047-1060.

55. Jennings, P.H., and T.A. Tattar. 1979. Effects of chilling on membrane potentials of maize and oat leaf cells. In, Low Temperature Stress in Crop Plants: The Role of the Membrane, J.M. Lyons, D. Graham, J.K. Raison, eds., Academic Press, New York, pp. 153-161

56. Jensen, R.D. and S.A. Taylor. 1961. Effect of temperature on water transport through plants. Plant Physiol. 36:639-642.

57. Jeschke, W.D. 1970. Lichtabhangige Veranderungen des Membranpotentials bei Blattzellen von Elodea densa. Z. Pflanzenphysiol. 62:158-172.

58. Jones, P.C.T. 1970. The effect of light, temperature, and anaesthetics on ATP levels in the leaves of Chenopodium rubrum and Phaseolus vulgaris. J. Exp. Bot. 21:58-63.

59. Keifer, D.W. and R.M. Spanswick. 1978. Activity of the electrogenic pump in Chara corallina as inferred from measurements of the membrane potential, conductance, and potassium permeability. Plant Physiol. 62:653-661.

60. Kelly, B. and E. Rubinstein. 1981. Light and oxygen stimulated proton efflux. Plant Physiol. 67:S-10.

61. Kinraide, T.B. and B. Etherton. 1982. Energy coupling in H^+-amino acid cotransport: ATP dependence of the spontaneous electrical repolarization of the cell membranes in oat coleoptiles. Plant Physiol. 69:648-652.

62. Kitasato, H. 1968. The influence of H^+ on the membrane potential and ion fluxes of Nitella. J. Gen. Physiol. 52:60-87.

63. Kuiper, P.J.C. 1970. Lipids in alfalfa leaves in relation to cold hardiness. Plant Physiol. 45:684-686.

64. Kuiper, P.J.C. 1972. Temperature response of adenosine triphosphatase of bean roots as related to growth temperature and to lipid requirement of the adenosine triphosphatase. Physiol Plant. 26:200-205.

65. Kuraishi, S., N. Arai, T. Ushijima, and T. Tazaki. 1968. Oxidized and reduced nicotinamide adenine dinucleotide phosphate levels of plants hardened and unhardened against chilling injury. Plant Physiol. 43:238-242.

66. Lewis, D.A. 1956. Protoplasmic streaming in plants sensitive and insensitive to chilling temperatures. Science 124:75-76.

67. Lin, W. 1979. Potassium and phosphate uptake in corn roots: Further evidence for an electrogenic H^+/K^+ exchanger and an OH^-/P_i^- antiporter. Plant Physiol. 63:952-955.

68. Lin, W. and J.E. Hanson. 1974. Increase in electrogenic membrane potential with washing of corn root tissue. Plant Physiol. 54:799-801.

69. Linden, C.D., K.L. Wright, H.M. McConnell, and C.F. Fox. 1973. Lateral phase separations in membrane lipids and the mechanism of sugar transport in Escherichia coli. Proc. Nat. Acad. Sci. 70:2271-2275.

70. Luttge, U., E. Ball, and K. von Willert. 1971. A comparative study of the coupling of ion uptake to light reactions in leaves of higher plant species having the C_3 and C_4 pathway of photosynthesis. Z. Pflanzenphysiol. 65:336-350.

71. Luttge, U. and N. Higinbotham. 1979. Transport in Plants. Springer-Verlag, New York.

72. Luzzati, V. and F. Husson. 1962. The structure of the liquid-crystalline phases of lipid-water systems. J. Cell Biol. 12:207-219.

73. Lyons, J.M., T.A. Wheaton, and H.K. Pratt. 1964. Relationship between the physical nature of mitochondrial membranes and chilling sensitivity in plants. Plant Physiol. 39:262-268.

74. Lyons, J.M. and C.M. Asmundson. 1965. Solidification of unsaturated/saturated fatty acid mixtures and its relationship to chilling sensitivity in plants. J. Amer. Oil Chem. Soc. 42:1056-1058.

75. Lyons, J.M. and J.K. Raison. 1970. Oxidative activity of mitochondria isolated from plant tissues sensitive and resistant to chilling injury. Plant Physiol. 45:386-389.

76. Lyons, J.M. 1972. Phase transitions and control of cellular metabolism at low temperatures. Cryobiology 9:341-350.

77. Lyons, J.M. 1973. Chilling injury in plants. Ann. Rev. Plant Physiol. 24:445-466.

78. Malek, F. and D.A. Baker. 1977. Proton co-transport of sugars in phloem loading. Planta 135:297-299.

79. Mandala, S.M. and L. Taiz. 1982. Proton and Cl^- transport in corn coleoptile vesicles. Plant Physiol 69:S-66.

80. Marre, E. 1979. Fusicoccin: a tool in plant physiology. Ann.Rev. Plant Physiol. 30:273-288.

81. Martens, J., U.-P. Hansen, and J. Warncke. 1979. Further evidence for the parallel pathway model of the metabolic control of the electrogenic pump in Nitella as obtained from the high frequency slope of the action of light. J. Membr. Biol. 48:115-139.

82. Martin, B., D.R. Ort, and J.S. Boyer. 1981. Impairment of photosynthesis by chilling temperatures in tomato. Plant Physiol. 68:329-334.

83. Margulies, M.M. 1972. Effect of cold-storage of bean leaves on photosynthetic reactions of isolated chloroplasts. Inability to donate electrons to photosystem II and relation to manganese content. Biochim. Biophys. Acta 267:96-103.

84. McMurchie, E.J. 1979. Temperature sensitivity of ion-stimulated ATPases associated with some plant membranes. In, Low

Temperature Stress in Crop Plants: The Role of the Membrane, J.M. Lyons, D. Graham, J.K. Raison, eds., Academic Press, New York, pp. 163-176.

85. McWilliam, J.R. and P.J. Ferrar. 1974. Photosynthetic adaptation of higher plants to thermal stress. Bull. Roy. Soc. New Zealand 12:467-476.

86. Melamed-Harel, H. and L. Reinhold. 1979. Hysteresis in the responses of membrane potential, membrane resistance, and growth rate to cyclic temperature change. Plant Physiol. 63: 1089-1094.

87. Mertz, S.M., Jr., and C.J. Arntzen. 1978. Depolarization of the electrogenic transmembrane electropotential of Zea mays L. by Eipolaris (Helminthosporium) maydis race T toxin, azide, cyanide, dodecyl succinic acid, or cold temperature. Plant Physiol. 62:781-783.

88. Miller, R.W., I. de la Roche, and M.K. Pomeroy. 1974. Structural and functional responses of wheat mitochondrial membranes to growth at low tmeperatures. Plant Physiol. 53:426-433.

89. Minchin, A. and E.W. Simon. 1973. Chilling injury in cucumber leaves in relation to temperature. J. Exp. Bot. 24: 1231-1235.

90. Nelles, A. and E. Laske. 1982. Temperature dependency of the membrane potential of corn coleoptile cells. Biochem. Physiol. Pflanzen 177: 107-113.

91. Nobel, P.S. 1974. Temperature dependence of the permeability of chloroplasts from chilling-sensitive and chilling-resistant plants. Planta 115:369-372.

92. Nolan, W.G. 1980. Effect of temperature on electron transport activities of isolated chloroplasts. Plant Physiol. 66:234-237.

93. Pallaghy, C.K. and U. Luttge. 1970. Light-induced H^+-ion fluxes and bioelectric phenomena in mesophyll cells of Atriplex spongiosa. Z. Pflanzenphysiol. 62:417-425.

94. Papahadjopoulos, D., K. Jacobson, S. Nir, and T. Isac. 1973. Phase transitions in phospholipid vesicles: Fluorescence polarization and permeability measurements concerning effect of temperature and cholesterol. Biochim. Biophys. Acta 311:330-348.

95. Pasternak, D. and G.L. Wilson. 1972. After-effects of night temperatures on stomatal behavior and photosynthesis of Sorghum. New Phytol. 71:683-689.

96. Patterson, B.D., T. Murata, and D. Graham. 1976. Electrolyte leakage induced by chilling in Passiflora species tolerant to different climates. Aust. J. Plant Physiol. 3:435-442.

97. Patterson, B.D. and D. Graham. 1977. Effect of chilling temperatures on the protoplasmic streaming of plants from different climates. J. Exp. Bot. 28:736-743.

98. Patterson, B.D., J.R. Kenrick, and J.K. Raison. 1978. Lipids of chill-sensitive and -resistant Passiflora species: Fatty acid composition and temperature dependence of spin label motion. Phytochem. 17: 1089-1092.

99. Paull, R.E., B.D. Patterson, and D. Graham. 1979. Amino acid uptake by tomato leaf tissue under chilling stress: Uptake conditions and a comparison of Lycopersicon species of different chilling resistance. Aust. J. Plant Physiol. 6:475-484.

100. Paull, R.E. 1981. Temperature-induced leakage from chilling-sensitive and chilling-resistant plants. Plant Physiol. 68: 149-153.

101. Pitman, M.G., S.M. Mertz, Jr., J.S. Graves, W.S. Pierce, and N. Higinbotham. 1970. Electrical potential differences in cells of barley roots and their relation to ion uptake. Plant Physiol. 47:76-80.

102. Poole, R.J. 1978. Energy coupling for membrane transport. Ann. Rev. Plant Physiol. 29:437-460.

103. Prins, H.B.A., J.R. Harper, and N. Higinbotham. 1980. Membrane potentials of Vallisneria leaf cells and their relation to photosynthesis. Plant Physiol. 65: 1-5.

104. Quinn, P.J. and W.P. Williams. 1978. Plant lipids and their role in membrane function. Prog. Biophys. Molec. Biol. 34: 107-173.

105. Raison, J.K., J.M. Lyons, and W.W. Thomson. 1971. The influence of membranes on the temperature-induced changes in the kinetics of some respiratory enzymes of mitochondria. Arch. Biochem. Biophys. 142:83-90.

106. Raison, J.K., J.M. Lyons, R.J. Mehlhorn, and A.D. Keith.

1971. Temperature-induced phase changes in mitochondrial membranes detected by spin labeling. J. Biol. Chem. 246:4036-4040.

107. Raison, J.K. 1974. A biochemical explanation of low-temperature stress in tropical and sub-tropical plants. Roy. Soc. New Zealand Bull. 12:487-497.

108. Raison, J.K. and E.A. Chapman. 1976. Membrane phase changes in chilling-sensitive Vigna radiata and their significance to growth. Aust. J. Plant Physiol. 3:291-199.

109. Rapoport, S.I. 1970. The sodium-potassium exchange pump: relation of metabolism to electrical properties of the cell. I. Theory. Biophys. J. 10:246-259.

110. Raschke, K. 1970. Temperature dependence of CO_2 assimilation and stomatal aperture in leaf sections of Zea mays. Planta 91:336-363.

111. Richardson, T. and A.L. Tappel. 1962. Swelling of fish mitochondria. J. Cell Biol. 13:43-54.

112. Saddler, H.D.W. 1970. The membrane potential of Acetabularia mediterranea. J. Gen. Physiol. 55:802-821.

113. Sheynour, A., J.K. Raison, and R.M. Smillie. 1973. The effect of temperature on the rate of photosynthetic electron transfer in chloroplasts of chilling-sensitive and chilling-resistant plants. Biochim. Biophys. Acta 292:152-161.

114. Smillie, R.M. and R. Nott. 1979. Assay of chilling injury in wild and domestic tomatoes based on photosystem activity of the chilled leaves. Plant Physiol 63:796-801.

115. Spanswick, R.M. 1972. Evidence for an electrogenic ion pump in Nitella translucens I. The effects of pH, K^+, Na^+, light and temperature on the membrane potential and resistance. Biochim. Biophys. Acta. 288:73-89.

116. Spanswick, R.M. 1972. Electrical coupling between the cells of higher plants: a direct demonstration of intercellular transport. Planta 102:215-227.

117. Spanswick, R.M. 1973. Electrogenesis in photosynthetic tissues. In , Ion Transport in Plants, W.P. Anderson, ed., Academic Press, New York, pp. 113-128.

118. Spanswick, R.M. 1974. Evidence for an electrogenic ion pump in

Nitella translucens II. Control of the light-stimulated component of the membrane potential. Biochim. Biophys. Acta 332:387-399.

119. Spanswick, R.M. 1981. Electrogenic ion pumps. Ann. Rev. Plant Physiol. 32:267-289.

120. Stewart, J.M. and G. Guinn. 1969. Chilling injury and changes in adenosine triphosphate of cotton seedlings. Plant Physiol. 44:605-608.

121. Stout, R. and R. Cleland. 1981. In vitro ATP-generated transmembrane electrical potential and pH gradient in oat root membranes. Plant Physiol. 67:S-9.

122. Sze, H. 1980. Nigericin-stimulated ATPase activity in microsomal vesicles of tobacco callus. Proc. Natl. Acad. Sci. 77:5904-5908.

123. Sze, H. and T.K. Hodges. 1977. Selectivity of alkali cation influx across the plasma membrane of oat roots: Cation specificity of the plasma membrane ATPase. Plant Physiol. 59:641-646.

124. Sze, H. and K.A. Churchill. 1981. Mg^{2+},KCl-ATPase of higher plant plasmalemma is an electrogenic pump. Plant Physiol. 67:S-43.

125. Taylor, A.O. and J.A. Rowley. 1971. Plants under climatic stress. I. Low temperature, high light effects on photosynthesis. Plant Physiol. 47:713-718.

126. Taylor, A.O., N.M. Jepsen, and J.T. Christeller. 1972. Plants under climatic stress. III. Low temperature, high light effects on photosynthetic products. Plant Physiol. 49:798-802.

127. Taylor, A.C., C.R. Slack, and H.G. McPherson. 1974. Plants under climatic stress. VI. Chilling and light effects on photosynthetic enzymes of sorghum and maize. Plant Physiol. 54:696-701.

128. Teeri, J.A., D.T. Patterson, R.S. Alberte, and R.M. Castleberry. 1977. Changes in the photosynthetic apparatus of maize in response to simulated natural temperature fluctuations. Plant Physiol. 60:370-373.

129. Thorne, J.H. 1982. Temperature and oxygen effects on ^{14}C-photosynthate unloading and accumulation in developing soybean seeds. Plant Physiol. 69:48-53.

130. Throm, G. 1970. Die lichtabhangige Anderung des Membranpotentials bei Griffithsia setacea. Z. Pflanzenphysiol. 63: 162-180.

131. Throm, G. 1971. Einflu von Hemmstoffen und des Redoxpotentials auf die lichtabhangige Anderung des Membranpotentials bei Griffithsia setacea. Z. Pflanzenphysiol. 64:281-296.

132. Throm, G. 1971. Aktionsspektrum der Photosysteme II und I fur die lichtabhangige Anderung des Membranpotentials bei Griffithsia setacea. A. Pflanzenphysiol. 65:389-403.

133. Throm, G. 1973. Untersuchungen zur Beziehung zwischen der lichtabhangigen und der redoxabhangigen Anderung des Membranpotentials bei Griffithsia setacea. Planta 112:273-284.

134. Treharne, K.J. and J.P. Cooper. 1969. Effect of temperature on the activity of carboxylases in tropical and temperate Gramineae. J. Exp. Bot. 20: 170-175.

135. van Eel, A.J.E. and A.J. van Erven. 1979. A model for proton and potassium co-transport during the uptake of glutamine and sucrose by tomato internode disks. Planta 145:77-82.

136. van Steveninck, R.F.M. 1975. The "washing" or "aging" phenomenon in plant tissues. Ann. Rev. Plant Physiol. 26:237-258.

137. Vredenberg, W.J. and W.J.M. Tonk. 1973. Photosynthetic energy control of an electrogenic ion pump at the plasmalemma of Nitella translucens. Biochim. Biophys. Acta 298:354-368.

138. Walker, N.A. and A.B. Hope. 1969. Membrane fluxes and electric conductance in characean cells. Aust. J. Biol. Sci. 22: 1179-1195.

139. Warncke, J. and C.L. Slayman. 1980. Metabolic modulation of stoichiometry in a proton pump. Biochim. Biophys. Acta 591:224-233.

140. Wilson, J.M. and R.M.M. Crawford. 1974. Leaf fatty-acid content in relation to hardening and chilling injury. J. Exp. Bot. 25: 121-131.

141. Wilson, J.M. 1978. Leaf respiration and ATP levels at chilling temperatures. New Phytol. 80:325-334.

142. Wolfe, J. 1979. Some physical properties of membranes in the phase separation region and their relation to chilling damage

in plants. In , Low Temperature Stress in Crop Plants: The Role of the Membrane, J.M. Lyons, D. Graham, J.K. Raison, eds., Academic Press, New York, pp. 327-335.

143. Wright, M. 1974. The effect of chilling on ethylene production, membrane permeability and water loss of leaves of Phaseolus vulgaris. Planta 120:63-69.

144. Wright, M. and E.W. Simon. 1973. Chilling injury in cucumber leaves. J. Exp. Bot. 24:400-411.

145. Wu, S.H. and H.M. McConnell. 1973. Lateral phase separations and perpendicular transport in membranes. Biochem. Biophys. Res. Comm. 55:484-491.